

Sixth European Conference of Apidology

Sixth European Conference of Apidology
9-11 September 2014

Edited by Pilar De la Rúa

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Monday 8		Tuesday 9		Wednesday 10		Thursday 11	
09:00-09:30	Welcome Address		Report Eurbee.org				
09:30-10:00	Plenary Dave Goulson		Plenary Ingolf Steffan-Dewenter		Plenary V.-L. Imperatriz-Fonseca		
10:30-11:00	Bee health	Pop. Gen. Physiology	Bee health	Pop. Gen. Physiology	Bee health	Stressors Chem. Ecol.	
11:00-11:30	<i>Coffee Break</i>		<i>Coffee Break</i>		<i>Coffee Break</i>		
11:30-13:30	Bee health	Pop. Gen. Physiology	Bee health	Pop. Gen. Physiology	Bee health	Stressors Chem. Ecol.	
13:30-15:00	<i>Lunch</i>		<i>Lunch</i>		<i>Lunch</i>		
15:00-16:00	Plenary Dennis vanEngelsdorp				Plenary Peter Rosenkranz		
16:00-17:00	Bee health	Pop. Gen. Genomics			Bee health	Stressors Bee products	
17:00-17:30	<i>Coffee Break</i>				<i>Coffee Break</i>		
17:30-18:30	Bee health	Pop. Gen. Neural basis	Social Event: visit to Cartagena		Bee health	Stressors Bee products	
19:30	Poster session/BEEHAVE workshop				Poster session		
20:30	Registration at Congress Venue				Banquet Hotel 7 Coronas		

Tuesday 9			
Miguel Angel Clares Hall		Hall 2	Hall 3
	Welcome Address		
09:00-09:30	Plenary Dave Goulson		
09:30-10:30	Peter Neumann	Andreas Wallberg	
10:30-10:45	Reinhold Siede	Alexey Nikolenko	
10:45-11:00		<i>Coffee Break</i>	Christian W.W. Pirk
11:00-11:30	<i>Coffee Break</i>		<i>Coffee Break</i>
11:30-11:45	Geoffrey Williams	Raffael Dall'Olio	Michael Hrnčir
11:45-12:00	Norman Carreck	Ameline Lehébel-Péron	Helmut Kovac
12:00-12:15	Kerstin Szonn	M. Alice Pinto	Anton Stabentheiner
12:15-12:30	Cristina Botías	Laura Jara	Karl Crailsheim
12:30-12:45	Jonathan Carruthers	Ralph Büchler	
12:45-13:00	Karina Antúnez	Evgeniya Ivanova	Anja Buttstedt
13:00-13:15	Antoine Lecocq	Andrzej Oleksa	Harmen P. Hendriksma
13:15-13:30	Juan Antonio Sánchez	Maéva A. Techer	Pierre Giovenazzo
13:30-15:00		<i>Lunch</i>	
15:00-16:00	Plenary Dennis vanEngelsdorp		
16:00-16:15	Mar Leza	Marina D. Meixner	Robert J. Paxton
16:15-16:30	Claudia Häußermann	Cecilia Costa	Matthew T. Webster
16:30-16:45	Paul Page	Maria Bouga	Jakrawut Maitip
16:45-17:00	Joel González-Cabrera	Axel Decourtye	Denise Aumer
17:00-17:30	<i>Coffee Break</i>		<i>Coffee Break</i>
17:30-17:45	Bettina Ziegelmann	Robin F.A. Moritz	Markus Thamm
17:45-18:00	Merav Gleit	Alexis Beaurepaire	Mary J. Palmer
18:00-18:15	Klemens J. Krieger	Irene Muñoz	Keram Pfeiffer
18:15-18:30	Antonio Nanetti	Naheed Rajper	Wolfgang Blenau
18:30-19:30	Poster session/BEEHAVE workshop		
	Advancing bee health	Pop. Genetics & Conserv.	Genomics
			Neural basis
	Advancing bee health	Pop. Genetics & Conservation	Physiology

Summary

Wednesday 10				
Miguel Angel Clares Hall		Hall 2	Hall 3	
09:00-09:30	Report Eurbec.org			
09:30-10:30	Plenary Ingolf Steffan-Dewenter			
10:30-10:45	Aleš Gregorc			
10:45-11:00	Anna Maria Gajda	Miguel Hurtado-Burillo Rodolfo Jaffé		
11:00-11:30	<i>Coffee Break</i>	<i>Coffee Break</i>		
11:30-11:45	Gina Retschnig	Denise Araujo Alves	Physiology	<i>Coffee Break</i>
11:45-12:00	Matthias Becher	Vanessa Bonatti		
12:00-12:15	Christoph Kurze	Juliana S. Galaschi Teixeira		
12:15-12:30	Raquel Martín-Hernández	Marina Lopes Grassi Sella		
12:30-12:45	Björn Dahle	Samuel Boff		
12:45-13:00	Daniela Arredondo	Kevin Maebe		
13:00-13:15	Irakli Janashia	Michael Lattorff		
13:15-13:30	Silvio Erler	Sandy Padilla	Reet Karise	
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18:15-18:30				
18:30-19:30				
<i>Lunch</i>				
Social Event: visit to Cartagena				

Thursday 11		
	Miguel Angel Claes Hall	Hall 3
09:00		
09:30-10:30	Plenary V.-L. Imperatriz-Fonseca	
10:30-10:45	Dirk C. de Graaf	
10:45-11:00	Fanny Mondet	Thomas Schmitt
11:00-11:30	Coffee Break	Coffee Break
11:30-11:45	Joachim de Miranda	Rita Cervo
11:45-12:00	Alexander E. Kalashnikov	Kaira Wagoner
12:00-12:15	Orlando Yañez	Francesco Nazzi
12:15-12:30	Esmail Amiri	Dirk Koedam
12:30-12:45	Sandra G. Mustafa	Yves Le Conte
12:45-13:00	Kitiphong Khongphinitbunjong	
13:00-13:15	Marie-Pierre Riviere	
13:15-13:30	Lotta Fabricius Kristiansen	Jevrosima Stevanovic
13:30-15:00		Lunch
15:00-16:00	Plenary Peter Rosenkranz	
16:00-16:15	Marie-Pierre Chauzat	Vassya Bankova
16:15-16:30	Jorgen Ravoet	Sangmi Han
16:30-16:45	Olivier Esnault	Per Kryger
16:45-17:00	Uros Glavinic	Coffee Break
17:00-17:30	Coffee Break	Miguel Vilas-Boas
17:30-17:45	Marco Pietropaoli	Ana Pascual-Maté
17:45-18:00	Alejandra Scannapieco	M. Teresa Sancho
18:00-18:15	Anja Amtoft Wynns	
18:15-18:30	Kirsten Foley	
18:30-19:30	Poster session	
from 20:30	Banquet Hotel 7 Coronas	

Preface

Every two years, members of the scientific community doing research in Apidology meet to exchange new knowledge about the fascinating world of bees.

This year, the European Congress of Apidology is taking place in Murcia, a small province on the Mediterranean coast of Spain, the country in Europe with the largest number of honeybee colonies (around 2.5 million).

The celebration of this congress has attracted the interest of researchers around the World, from Brazil to South Korea and from Sweden to South Africa. We expect this event to be a hotbed of new ideas and projects that will enable us to move forward in combating the widespread decline of pollinators. Indeed, overall concern about pollinators loss and the drivers causing it, has spread all over the World, and society is waiting for researchers to find appropriate measures to solve this problem. We also hope that a good trade off between senior and young researchers and the media will happen during this event, to find ways to conserve and improve these vital insects for our ecosystems.

But science does not oppose amusement. We are in a city celebrating its September fiesta, open to share many events, drinks and tapas with all visitors. You are therefore more welcome than ever to Murcia. Enjoy it, and enjoy the meeting!

Pilar De la Rúa
President of Eurbee

Acknowledgements

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And also to our collaborators: Campus Mare Nostrum, Centro de Edafología Aplicada del Segura (CEBAS-CSIC), Murcia Convention Bureau.

Plenaries

Impacts of pesticides and other stressors on bee health

Prof Dave Goulson

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The causes of pollinator decline, and in particular the relative contribution of pesticides in driving these declines, remains controversial. Here I will provide a review of the evidence, with a particular focus on neonicotinoids and their impacts on bumblebee colonies. A coherent and consistent body of research demonstrates that field-realistic exposure is likely to be causing significant harm to wild bumblebee populations through elevated worker mortality and a range of sublethal effects including reduced pollen gathering and reduced fecundity, which together reduce colony success. Stress induced by neonicotinoids does not act in isolation; bees are likely to be simultaneously and chronically exposed to multiple pesticides including pyrethroids and DMI fungicides, the latter acting synergistically with insecticides. Bees also face challenges from novel pathogens and parasites, and neonicotinoids impair their immune system and so exacerbate the impacts of these agents. The complex interactions between dietary stress, diseases and pesticides exposure, and the difficulties of controlling these in the field, make it hard to perform convincing field experiments with free flying bees, posing a great challenge to the ingenuity of researchers. I will finish by considering the broader environmental concerns raised by current practices in pest management, which have largely abandoned the long-established principles of Integrated Pest Management (IPM), and rely instead on prophylactic use of persistent, systemic chemicals.

Factors effecting colony health in the US

Dennis vanEngelsdorp

Department of Entomology, University of Maryland, United States of America

High levels of loss have been the norm for US beekeepers over the last 7 years. The underlying drivers for these annual losses have been much debated. Here we take a look at what is generally thought of as the three leading contributors to the losses; Varroa mites, pesticides and poor nutrition. The evidence for each will be reviewed and their likely contribution put in context. For instance, the National Honey Bee Disease survey and other survey efforts have documented varroa mite levels and pesticide incidence in the US which allow us to make more informed assessment of the impact of these factors may have on colony health. Evidence for synergistic effects between these factors will also be considered.

Interestingly, while annual losses have been high over the last 8 years, the actual number of managed colonies in the country has increased. Separately we will consider the factors driving this increase, focusing on socio-economic drivers and beekeeping practices.

Impact of land use change on bee diversity and crop pollination services

Ingolf Steffan-Dewenter

Department of Animal Ecology and Tropical Biology, University Würzburg, Germany

Global change, comprising habitat loss and fragmentation, land use intensification, climate change and species invasions, threatens biodiversity and the maintenance of ecosystem services. Focusing on pollination as an important ecosystem service I will explore the relative importance of individual drivers and their combined effects on functional diversity, relevant spatial and temporal scales, the dependence of responses on life history traits and the importance of species-richness for the resilience and stability of ecosystem services. I will also address the potential economic and societal consequences of disrupted ecosystem services and mitigation options in managed ecosystems.

Pollination services of neotropical bees: Trends in a rapidly changing world.

Vera Lucia Imperatriz-Fonseca

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Bees are important to deliver pollination services, being part of the reproduction, seed formation and genetic diversity of plant species. During the last two decades, evidence of pollinator declines has accumulated and multiple drivers have been identified as causes. These declines may have important consequences for food production, since many bees species are pollinators of agricultural crops. Climate change could jeopardize the interaction between plants and pollinators, since climate regulates the physiology and phenology of both plants and bees. For instance, species distribution models have already shown that climate change will alter the distribution of some species, which are expected to seek cooler and higher locations. Consequences of this, especially to crop production, are yet to be addressed. In addition, habitat loss and fragmentation have been identified among the major drivers of pollinator declines, as changes seem to occur at a higher speed than that needed by species to adapt to these changes. As study case we used stingless bees species of extreme climate occurrence, that may be used as reference for monitoring, management and protection during changing times to come.

Parasite-host-interactions during the reproductive cycle of the honey bee mite *Varroa destructor*

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The honey bee mite *Varroa destructor* represents the greatest threat for apiculture and is considered the main factor for colony winter losses in temperate climates. The main reason for the host damages in *Apis mellifera* is the unlimited growth of the mite population during the season. Varroa population modellings suggest that in this respect the reproductive success of the female mite within the brood cells is of crucial significance. It should therefore be an adaptive strategy for the host to restrict the reproductive success of the parasite. This is confirmed not only by the original host *Apis cerana* but also by some *A. mellifera* populations that have survived over long periods without any Varroa treatment. The presentation will focus on how host factors might disturb the reproduction of the Varroa females. Reproducing Varroa females are under a tremendous time pressure caused by the relatively short capping period of the honey bee brood. During this period at least one male and female offspring must reach the adult stage and mate before the host bee hatches. We present experiments that demonstrate how the female mites synchronize their reproductive cycle with stage specific signals from the host larvae. Importantly, these host signals can both, activate and interrupt the oogenesis of the mite. Volatile compounds of the larval cuticle are, at least in part, involved in this host-parasite interaction. The chemical nature of these compounds and the possible impact for biological control and selective breeding are discussed.

Spoken presentations

Advancing Bee Health

Organizers: Raquel Martín-Hernández, Geoffrey Williams

Promoting the health of bees is essential to preserve biodiversity and to ensure food security via pollination. Recently bees have been confronted with serious challenges that have compromised their viability in many regions of the world. To ensure an adequate and sustainable bee sector, cooperation among researchers is essential. This symposium will focus on encouraging the well-being of both *Apis* and non-*Apis* bees. Topics to be included are novel pest & parasite threats to bees, land-use impacts, and nutrition, among others, with the hope of developing new foci for research that will promote sustainable, healthy bee populations.

Invited speakers: Dirk de Graaf, Ales Gregorc, Mari-Pierre Chauzat, Peter Neumann, Joachim de Miranda

Neonicotinoid exposure overrides the impact of a parasite during hibernation of bumblebee queens, *Bombus terrestris*

Aline Fauser-Misslin, Ben M. Sadd, Christoph Sandrock, Peter Neumann

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Seasonal adaptations are widespread among organisms, enabling the bridging of periodic challenging conditions. However, while moderated, these periods often remain sensitive. Several temperate insects with vulnerable over-wintering stages, including many pollinators, play key ecosystem and economic roles. Due to their ecosystem and economic roles, recent declines of wild bees have raised considerable concern. Various stressors, including pesticides and parasites, alone or in combination, have been implicated. In temperate bumblebees, *Bombus* spp., only new queens of social colonies overwinter, and hibernation survival is crucial for population viability. We investigate how dietary exposure to field-realistic neonicotinoid insecticides (thiamethoxam and clothianidin) and/or the prevalent parasite *Crithidia bombi* prior to hibernation affects hibernation survival of *Bombus terrestris* queens. Exposure to the neonicotinoids and *C. bombi* both reduce hibernation success in isolation. Effects are asynchronous, however, and in the presence of both stressors early pesticide effects mask later parasite effects. Despite this masking, our results demonstrate that neonicotinoid exposure will, at the population level, exacerbate negative effects of parasites and have a detrimental impact on long-term population viability. This highlights the importance, yet vulnerability, of resistant life stages employed by numerous organisms to overcome seasonal challenges.

Testing Thiaploprid: Does a long-term exposure affect bee colonies?

Reinhold Siede, Lena Faust, Marina Meixner, Christian Maus, Bernd Grünwald, Ralph Büchler

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The neonicotinoid thiacloprid is registered for use in flowering crops. Bees might get in contact with the substance. Potential effects of a long-term exposure have caused concerns. To assess that assumption a field trial with 30 bee colonies was undertaken in Germany from 2011 to 2014. The colonies were divided in three groups (C, T1, T2) of ten colonies each with colony and year as replicates. The colonies were started as shook swarms in July of each year. They were arranged in an experimental yard. From July to October control group C was fed with 25 l pure syrup per colony. The treatments T1 and T2 were provided with syrup spiked with 200 ppb or 2000 ppb thiacloprid resp. . Colonies were weighed and the numbers of bees were estimated in intervals of 21 days according to the Liebefeld method. Dead bees were counted regularly. Infestation with Varroa und Nosema was measured. A preliminary evaluation of the data revealed small effects of the

factor group, but high effects of the factor year. Mean numbers per year were I) for bees: 15550, 9607, 12680 (group C); 14420, 10002, 14145 (group T1) and 13818, 9051, 12588 (group T2) and II) for brood: 13767, 10115, 6152 (group K), 12851, 10259, 6507 (group T1) and 11960, 9699 and 6547 (group T2). Differences between groups were not significant ($p > 0.05$, linear mixed model, spps, v 19). Chronic exposure to thiacloprid did not affect the performance of the colonies.

God Save the Queen: influence of agro-chemicals on honey bee queen physiology and behaviour

Geoffrey Williams, Aline Troxler, Gina Retschnig, Kaspar Roth, Dave Shutler, Orlando Yañez, Peter Neumann, Laurent Gauthier

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In monogynous species of eusocial hymenoptera such as the honey bee, *Apis mellifera*, queen fate is a key element of colony survival and productivity. In both Europe and North America poor queen health appears to be a major driver of honey bee colony mortality; however, most studies investigating colony health have so far concentrated on workers. Here we exposed developing queens to sub-lethal field-realistic doses of agro-chemical pesticides and recorded multiple physiological and behavioural measures in the laboratory and field over a four week post-emergence period in which queens were maintained in mating nucleus colonies ($N = 30$ & 28 for treatments and controls, respectively). The data show that the pesticides had no significant influence on queen lifespan, emergence weight, spermathecal sperm viability, or number of flights, whereas they significantly affected queen success (i.e. production of worker brood progeny), spermathecal sperm count, and ovariole number. These results demonstrate that sub-lethal exposure to agro-chemicals can have a profound effect on honey bee queens, and may at least partially help to explain the recent phenomenon of reduced queen health experienced by many beekeepers internationally.

Bee visitation on neonicotinoid treated maize in the UK

Norman Carreck, Francis Ratnieks

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A number of studies have been published on the neonicotinoid contents of seed treated maize crops and the possible effects on foraging bees. In contrast to the situation in many other countries, maize is not normally thought to be a major forage source for bees in the UK. We studied pollen collection by honey bees from a farm which is the largest producer of maize (sweetcorn) for human consumption in the UK. This maize, all of which

was treated with thiamethoxam seed dressing, flowered late in the season when few other forage sources were available to bees. The results in terms of plant and pollen neonicotinoid contents will be discussed in relation to other pollen sources available and the nutritional limitations of maize as a pollen source for bees.

Development of a Toxicokinetic-Model of the Bee Hive

Kerstin Szonn¹, Christian Maus², Alexander Nikolakis², Hans-Toni Ratte³, Martina Roß-Nickoll¹, Walter Schmitt⁴, Thomas G. Preuss^{1,2}

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Pollination has to be considered as an important factor in food economy and the honey bee *Apis mellifera* is the most important commercial pollinator. Over the last few years, potential effects of insecticides on bee colonies' health have been discussed. There is largely consensus that the risk assessment for bees will benefit from a deeper understanding of mechanisms of bee exposure to pesticides and to understand the effects of toxicants in the bee hive it is important to fully understand their fate within bee colonies.

We want to tackle this question with the calculation of the substance distribution in the bee hive and choose the modelling approach of a Physiologically Based Toxicokinetic Model inspired TK-Model to describe the absorption, distribution and excretion of substances in the bee hive. A conceptual model, which is inspired by PBTK-models, was developed; it comprises all relevant processes. It is the basis for a model simulating bee hive toxicokinetics. This model is implemented in the programming language R and will provide a deeper understanding about toxicokinetics and exposure in the bee hive. It will predict internal concentrations of bees. It shall bring insight to the question of how to link pesticide exposure to potential effects within the colony and onto colony health.

No way out for bees: Residues of neonicotinoids present not only in flowering crops, but also in wildflowers

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Neonicotinoid insecticides are now among the most widely used pesticides in the world, and are routinely used to protect a range of flowering crops. Most are used as a seed-dressing, with the crop plant absorbing the compound as it grows, and hence becoming protected against herbivorous pests. Neonicotinoids are also used as foliar sprays on soft fruit crops, and are widely sold as sprays or granules for garden use on flowers, fruit

and vegetable crops. As they are systemic chemicals, low concentrations of neonicotinoids are found in nectar and pollen of treated crops, and hence they are consumed by pollinators. They are also water soluble and persistent, so that detectable quantities can be found in field margin wildflowers near treated crops, although it is not known if this commonly occurs. The present study reports the concentrations of pesticides found in pollen from different plant species brought back to the colonies by honeybee foragers (pollen traps), in pollen stored in bumblebee nests, and in pollen directly collected from the flowers of treated crops (oilseed rape) and wild flowers from the field margins of crops. The results reveal the presence of neonicotinoid insecticides not only in the treated plants, but also in the wild flowers, which are major forage sources for both managed bees and wild pollinators in arable landscapes. Persistence of neonicotinoids in the environment and their occurrence in non-target vegetation are of concern as there is clear evidence that exposure to field-realistic levels of neonicotinoids has significant sublethal impacts both in social and solitary bees.

Crop cultivars and health of pollinator populations

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A key driver of pollinator declines in farmland is the scarcity of flowers to provide dietary pollen and nectar. Planting of mass-flowering crops enhances the floral resources available to pollinators during part of the year, which may improve their fitness in the surrounding landscape. But little is known about how modern cultivars of mass-flowering crops differ in the nutritional resources they produce, and the consequent value of these to pollinators.

The nutritional value to pollinators was compared between different cultivars of oilseed rape (*Brassica napus* L.), and short rotation coppice willow (*Salix* species). Both crops provide pollen and nectar used by *Apis* and non-*Apis* bees in early spring.

Pollen and nectar were collected and measured from a range of cultivars of both crops. High Pressure Liquid Chromatography was used to elucidate the composition of amino acids in the pollens, and the sugars and amino acids in the nectars of the different cultivars. A combination of feeding experiments and modelling was then used to test how the cultivars' nutritional resources were related to fitness in bumblebees.

These findings show the influence of cultivar selection by growers upon the health of pollinator populations.

Pollen diversity is associated to honeybee health

Karina Antúnez, Matilde Anido, Belén Branchiccela, Jorge Harriet, Juan Campá, Ciro Invernizzi, Estela Santos, Mariano Higes, Raquel Martín-Hernández, Pablo Zunino.

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Honeybee's ability to resist infection by different parasites and pathogens is compromised if they are malnourished or exposed to pesticides. The aim of the present work was to evaluate the interaction between pollen diversity and honeybee parasites and pathogens, without the influence of agricultural chemical contamination. We monitored 40 colonies in two apiaries along 24 months, regularly monitoring sanitary status and pollen diversity. No agrochemicals were found in pollen in any colony or in any season. *Varroa destructor* was present in all colonies in March (autumn), decreasing after acaricide treatments. ABPV, DWV and SBV presented a similar trend; being associated to the mite. IAPV and KBV were not detected. *Nosema ceranae* was present along the whole year, being the main microsporidia detected. Colonies with access to pollen of diverse botanical origins, especially in autumn and winter, showed reduced infection levels of ABPV, BQCV, DWV, SBV and *N. ceranae* in the same season and following months. Evenmore, we found that higher concentration of pollen from *Bacharis trimera*, *Trifolium pretense*, *Trifolium repens* and *Senecio brasiliensis* during autumn - winter, decreased viral infection levels. These results confirm that an adequate nutrition is essential for the development of healthy colonies.

Weight watching and the effect of landscape on honeybee productivity: Insights from colony scale data in Denmark

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Over the last few decades we have witnessed a transformation from traditional agricultural practices, influencing the landscapes they occupy. In the face of such change, monitoring the development and productivity of honey bee colonies from different sites can give valuable insight on the influence of landscape type on their productivity and might point towards future directions for modernised beekeeping practices. In Denmark, the Danish Beekeeper's Association (BIAVL) fitted colonies around the country with electronic scales, sending regular weight data, to a mainframe database. Once we had cleaned and extracted relevant information from the data provided by BIAVL, we investigated the effect of the immediate landscape on colony productivity in Denmark. Preliminary results show that colonies in urban areas were significantly more productive than colonies in mixed and agricultural areas. This study serves not only as a start-up point to promote the systematic installation of scales in apiaries, but also as more evidence that 'city bees' are consistently doing better than those in agricultural landscapes.

The effect of increasing plant diversity in field margins on the abundance and diversity of bees

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The transformation of natural ecosystems by the intensification of agriculture has negative effects on bee communities. “Operation Pollinator” aims to increase field diversity to enhance wild bee habitats. The assays were carried out during 2011 and 2012 in Almería (Spain). Edges of natural vegetation were created along margins of intensively managed vegetable fields. Two types of edges were tested: (1) shrubby plants, integrated mainly by Lamiaceae species, and (2) herbaceous plants from several families. Bees were sampled using yellow pan traps. Most of the bees collected in traps were *Apis mellifera* (42.6%), *Eucera* (22.3%), *Lasioglossum* (*Lasioglossum*) (15.3%), *Andrena* (10.6%), *Panurgus* (3.9%), *Lasioglossum* (*Evylaeus*) (3.6%) and *Halictus* (1.2%). The number of captures and bee species were similar in herbaceous and the shrubby edges. Edges of herbaceous and shrubby plants produced an increase in the number of certain groups of bees (e.g. *Lasioglossum*, *Andrena*, *Panurgus* and *Halictus*) in relation to non-revegetated fields. Revegetated edges had an impact in the structure of the bee community, increasing the number of taxa and diversity. Flowering edges were highly used by *A. mellifera* and, thus, the revegetation of field margins may also translate into benefits to this domestic bee. This margins might also contribute to increase ecosystem services.

Impacts of host, climate, landscape and management on *Varroa destructor* abundance in Western Mediterranean.

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Rapid and substantial losses have been observed in honeybee populations worldwide over the last decade suffered. These losses have been attributed to a combination of different potential causes, such as diseases (*Varroa destructor*, *Nosema ceranae*), poor nutrition and frequent applications of insecticides to control pests. The mite *V. destructor* is currently one of the greatest menaces for apiculture. Mite population growth is variable and depends on multiple internal factors. However, microclimatic conditions in the hive are affected by external factors that could indirectly impact on mite populations.

The present study investigated the role of host density, natural resource availability for hosts, management and climate in driving spatial and annual variability in abundance of *V. destructor* recorded in apiaries across a Mediterranean island over a two-year period, using a hierarchical generalized linear model framework.

The best performing models combined different types of variables, including biological, landscape, climatic and management factors. Mite abundance decreased as the proportion of natural resources in surrounding landscapes increased and also as a result of anti-varroa treatments but increased as bee abundance increased. A preference for low ambient temperatures was observed in the first half of the year and on a monthly basis. Mite abundance in the second half of the year was negatively affected by humidity levels. These empirical environmental relationships are valuable for integration into bee and mite population model frameworks and also impact optimal control strategies for apiculture in different landscapes.

The effect of mating and spermiogenesis on the reproduction of female *Varroa destructor*

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Mating of *Varroa destructor* takes place inside the sealed honey bee brood cell. During copulation male mites transfer the sperm cells via a spermatophore into the gonopores of the females. The number of transferred sperm cells has to be sufficient for the whole life span of the female mites. Thus mating of *V. destructor* strongly influences the following reproductive cycles of female mites. We first recorded the percentage of unmated *Varroa* females within the phoretic mite population. Furthermore we compared the reproductive capacity of mated and unmated female mites that have been artificially reared. In the mated *Varroa* females we analyzed the spermiogenesis by dissecting the genital tract in regular time intervals. We define certain steps of the spermiogenesis and present a time course from the transfer of the spermatophore till the first presence of mature sperm cells. We therefore confirm that the sperm cells have to mature inside the spermateca of the female before a fertilized egg can be laid. We proved this by introducing freshly mated *Varroa* females of different age into capped brood cells and recorded their fertility and fecundity 10 days later. Our results indicate that freshly mated female mites are able to reproduce without a phoretic phase. Obviously, the time span between mating of a female mite and the first oviposition of a fertilized egg is sufficient for the maturation of the transferred sperm cells.

Host specificity in the *Varroa* spp. – *Apis cerana* system

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The ectoparasitic mite *Varroa destructor* shifted from its original host, the Eastern honey bee, *A. cerana*, to the Western honey bee, *Apis mellifera* and is now one of the major biotic threats to *A. mellifera* beekeeping globally. Previous studies have shown that host specificity exists between *A. cerana* populations and their associated *Varroa* haplogroups, which is relevant for our understanding of host specificity and ultimately of the *V. destructor* host shift to *A. mellifera*. However, the biogeography of this host-parasite association was elucidated based on mites of unknown reproductive status. In three distant locations within Thailand, we readdress this issue by genetically identifying mites that are capable of effectively reproducing on local *A. cerana* lineages. The potential of colony infestation by more than one mite haplogroup was also investigated, relying on a large sample size within location and within colony. This approach will allow us to define host specificity at a higher resolution, providing us with a solid basis to unravel the biological mechanisms underlying the capacity of some *V. destructor* haplogroups to successfully reproduce on *A. mellifera*.

A target site mutation causes resistance to pyrethroids in European populations of *Varroa destructor*.

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The ectoparasitic mite, *Varroa destructor*, has played a prominent role in the decline in bee colony numbers over recent years. Pyrethroids like tau-fluvalinate and flumethrin can be highly effective in removing the mites from hives; but their intensive use has led to many reports of resistance.

To investigate the mechanism of resistance, the transmembrane domain regions of the *V. destructor* voltage-gated sodium channel (the main target for pyrethroids) were PCR amplified and sequenced from pyrethroid treated / untreated mites collected at several locations in Central/Southern England. A novel amino acid substitution, L925V, was identified mapping in a region proposed to form part of the pyrethroid binding site, also a known “hot spot” for resistance.

Using a high throughput diagnostic assay capable of detecting the mutation in indi-

vidual mites, the L925V substitution showed a strong correlation with resistance, being present in all mites that had survived tau-fluvalinate treatment but in only 8 % of control, untreated samples. Further analyses showed that the mutation is present in samples collected in several European countries, but only with high frequency in apiaries regularly treated with pyrethroids. This diagnostic assay should be a valuable tool to develop a pro-active monitoring programme aimed to design a resistance management strategy that, rotating different acaricides (including pyrethroids), maintains a more effective control of this highly damaging pest.

Mating disruption of *Varroa destructor* under colony conditions: a first approach for a biological mite control.

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Mating of the honeybee mite *Varroa destructor* takes place within the sealed host brood cell between the male and usually the youngest adult daughter. In previous studies we showed that the male's preference behavior is triggered by a female sex pheromone consisting of three fatty acids and the respective ethyl esters. In a laboratory bioassay we proved that male mites were not able to distinguish between receptive female and either older or immature females when exposed to an overdose of the sex pheromone. In order to examine this effect on the colony level, an empty brood comb was treated with an alcoholic pheromone solution while a control comb was treated with the solvent only. The queen was caged on the treated combs and freshly capped brood cells were artificially infested with phoretic *Varroa* females. After 11 days, the infested brood cells were opened and the adult daughter mites were removed and dissected. The number of spermatozoa in the spermathecae of these females was compared with the number of spermatozoa in daughter mites taken from the control comb. We confirmed that the number of spermatozoa was significantly reduced in *Varroa* females from the treated brood comb and a substantial number of these females didn't have spermatozoa at all. Although we could not completely hinder males from mating, we could reduce the number of successful copulations under colony conditions. This should have a significant impact on the mite's population dynamic. Our results therefore represent a promising step toward a biological *Varroa* control.

RNAi to control *Varroa* mite

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It is now generally accepted that several factors contribute to the worldwide decline

of honey bee health. The *Varroa* mite (*Varroa destructor*) is of particular significance. In many studies it was shown that high *Varroa* mite levels lead to colony decline. *Varroa* is the number one pest of managed honeybees (*Apis mellifera*) and a serious global threat to commercial beekeeping.

Beekeepers use several types of products against *Varroa*, including chemical miticides, organic acids and essential oils. However, due to the development of resistance many of these pesticides have lost their efficacy. The search for safer and more durable alternatives is ongoing.

RNA interference (RNAi) is a natural mechanism discovered in the late 1990s by Andrew Fire and Craig Mello, earning them the Nobel Prize in 2006 for this achievement. This mechanism is used by different organisms for defense from predators and regulation of gene expression and is based on a highly specific sequence recognition process. RNAi can be used to precisely target and suppress critical genes, leading to a specific pesticidal effect.

Using in-vitro assays we demonstrate that *Varroa*-specific dsRNA can reduce the target gene expression and increase *Varroa* mortality, without effecting bee survival. Moreover, we show that by targeting bee viruses we can reduce virus replication and increase bee survival in lab experiments.

The *Varroa*-Gate – a joint Effort of Academia and Pharmaceutical Industry for a sustainable *Varroa* Control

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Varroa destructor and *varroa* transmitted infectious diseases still pose the biggest challenge for bee health. Acaricides, biotechnical methods, essential oils and organic acids have been developed for *varroa* control, however, so far all methods have limitations, i.e. resistance, residues or side effects, and average annual losses of bee colonies continue to stay high. The molecular mechanism of *varroa* resistance to synthetic pyrethroids is already well understood and prevalence can be monitored by means of qPCR and bio assays. Further resistance selection can be avoided by the implementation of integrated pest management principles (IPM). Acaricide residues in honey are always a consequence of wax contamination, and side effects are a result of low safety margins and irregular exposure of bees to active ingredients. Both, residues and side effects can be avoided by following good bee keeping practice principles and by innovative formulation and application technologies.

Novel polymer matrix acaricide carrier with different pharmacodynamics have been developed for rotational treatment and prevention of *varroa* infestation for up to four months after honey extraction. Results from four clinical studies which were performed under different conditions of use within Europe confirm excellent efficacy and safety.

Clinical and residue data obtained so far suggest further development and regulatory approval. The so called *Varroa-Gate* is considered an innovative option for sustainable varroa control within an integrated pest management concept.

Performance of sugar dusting in predicting the infestation level of *Varroa destructor*

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The idea of assessing *Varroa destructor* population in a honeybee colony by dislodging the mites with powdered sugar on a small sample of workers is about ten years old.

Recently this practice has become popular in various contexts as a tool for the infestation quantification and treatment planning.

Here we evaluate the performance of the method in predicting the level of infestation in a colony.

On day one a sample of approximately 150 bees has been taken from an outer comb, they have been dusted with two tablespoon of powdered sugar and, after separation of the sugar, the mites were counted.

Every sample of bees underwent a soapy water wash to observe any remaining mite.

The same day the queens have been caged and the colonies have been treated to assess the number of phoretic mites.

After 25 days of caging, the queens were released and all the colonies underwent another treatment to evaluate the mites left.

The data obtained from the sample failed to reach a good correlation with the total population of mites and with the phoretic mites as well.

It is noteworthy that the powdered sugar showed poor efficiency also in estimating the mites in the sample, seeing the number of them recovered with the wash in soapy water.

Therefore we think that this practice may conduce to misleading assessments of infestations and incorrect decisions on treatment need and, therefore, it should not be used.

Instead we suggest the use of scheduled treatments, regardless the infestation level.

Pesticides and pathogens synergize the effect on honey bee

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Pathogen–pathogen interaction is known for *Varroa destructor* and is associated with different viruses and pathogen. Together they may have a significant impact on individual honey bees and on the overall colony. Potential interactions between pesticides and pathogens are poorly understood. Sublethal effects of various pesticides on bees lead to

alterations in cellular physiology, consistent with chemically induced stress. Physiological responses of bees to chemical and biological threats was measured by gene expression, after exposure to parasitic mite and pesticide and evaluated by changes on tissue level. Impacts of pesticides and Varroa parasitism on honey bee larval gene expression were demonstrated and transcript levels for different proteins, was significantly upregulated or downregulated. In experimentally infected winter bees, inoculated *per os* (*p/o*) or per cuticle (*p/c*) with CBPV, we evaluated the virus replication and studied potential synergistic effects when bees were co-infected with CBPV and *Nosema ceranae* (*N. ceranae*). CBPV replication was less effective when bees were inoculated *p/c* in comparison to *p/o* inoculation. Co-infection of workers with CBPV and *N. ceranae* showed an increased replication ability for CBPV. The highest level of bee mortality was observed in bees inoculated with CBPV *p/o*, followed by workers simultaneously inoculated with CBPV and *N. ceranae* *p/o*. The lowest mortality rate was observed in *N. ceranae* inoculated bees. The simultaneous infection with CBPV and *N. ceranae* induced a synergistic effect in reduced lifespan. Interactions between larval treatments and gene expression for the targeted genes and survival of CBPV and *Nosema* inoculated bees are discussed.

***Nosema ceranae* in interactions with *Nosema apis* and Black Queen Cell Virus**

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Nosema ceranae is a new parasite of *Apis mellifera* and there is still little information about its interactions with other pathogens, for instance about simultaneous infection of *Apis mellifera* by *N. ceranae* and *N. apis*, and about the influence of BQCV on type C nosemosis. We performed cage tests in which individual bees (5 days old) were infected with the mentioned pathogens in different combinations. Bees infected with both *Nosema* species (singular and in mixed infection) started to die on the 14th day p.i. The number of *N. apis* DNA copies in bees that died was constant regardless of the time p.i., whereas the *N. ceranae* DNA copy number increased over time. Bees with *N. ceranae* and BQCV lived significantly shorter than with *N. ceranae* alone. The virus multiplied more intensively in bees infected with *N. ceranae* than in infected with *N. apis*. In bees that died of the infection with *N. ceranae* and BQCV the number of *Nosema* spores was significantly lower than in bees infected with *N. ceranae* alone, which might be due to a shorter life span of the first. This tendency was not observed in the case of *N. apis* and BQCV infection. The results suggest that: in mixed infection *N. ceranae* is more efficient in colonization of the midgut than *N. apis*; infection of *N. ceranae* and BQCV leads to much faster death of bees than pure *N. ceranae* infection; and also that *N. ceranae* infection favors the infection with BQCV more than *N. apis*.

Stressors in perspective: impact of pesticides, parasites and transportation on honey bee (*Apis mellifera*) workers

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Interactions between pesticides and parasites may constitute one mechanism responsible for increased mortality of managed honey bee (*Apis mellifera*) colonies in the northern hemisphere. Because previous studies were mostly conducted in the laboratory, we investigated potential effects under more natural conditions in observation hive colonies. In a crossed experimental design, we challenged worker honey bees (N=1188 per treatment group) that originated from the same source colonies (N=8) in two distant locations (~300 km) with sublethal doses of the commonly applied neonicotinoid pesticide thiacloprid and the miticide tau-fluvalinate, as well as with ~100,000 spores of the ubiquitous endoparasite *Nosema ceranae*, and observed them in observation hives during 14 days. Results did not suggest synergy among stressors, but revealed rather weak effects of the pesticide and parasite exposure on both honey bee mortality and behaviour at the two study sites. However, regardless of treatment the data clearly show a reduced longevity of adult bees that were transported during the pupal stage to the second study site. This suggests that migratory beekeeping, whereby colonies are transported from one location to another, can have a strong effect on worker lifespan.

Foraging efficiency driving the impact of stressors on honeybee (*Apis mellifera*) colony performance: insights from the integrated model BEEHAVE?

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The decline of managed honeybee colonies in Europe and USA is of increasing concern. Although the underlying causes remain unclear, a combination of stressors, e.g. parasites, pathogens, pesticides, and changes in forage availability due to land use change, might cause the observed increased mortality. We used the integrated model BEEHAVE to explore scenarios which combined the effects of various foraging conditions such as foraging distance, timing of ‘hungry gaps’, and timing of pesticide exposure leading to increased forager mortality, in a stylized landscape providing a single food source.

We found that colony size and honey stores at the end of the year is most sensitive to hungry gaps exceeding a period of three weeks in June, July, or August even at low foraging distances, whereas for hungry gaps up to 30 days in April similar effects only occurred if foraging distance was increased to 3000 m. However, if exposed to pesticide-induced

doubled forager mortality over 30 days in June, hungry gaps longer than 21 days in July led to a reduction in colony size between 19% and 100% (at 500 m and 1000 m distance to the food source) even under ideal food supply.

Our results show how one stressor can increase the vulnerability to a second stressor, and that a colony's sensitivity to stressors is influenced by the timing of imposed stress relative to the colony dynamics at that time. Future model analyses have to focus on realistic landscape settings, with multiple food sources and their spatio-temporal dynamics.

Escaping parasite manipulation: Apoptosis and host-parasite co-evolution in *Apis mellifera*

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Programmed cell death (apoptosis) does not only play an important role in the development of multicellular organisms, but also in the protection against pathogens. Nevertheless, numerous intracellular pathogens have evolved diverse strategies to interfere with and overcome the apoptotic machinery of their hosts. Yet, little is known about the actual mechanisms and how hosts might counter act. We here study the interaction of the intestinal microsporidian parasite *Nosema ceranae* in a susceptible and tolerant honeybee host under laboratory controlled conditions, to understand the importance of apoptosis in this case of host-parasite co-evolution. We visualize apoptotic processes in the gut epithelium using TUNEL assays; relate this to the expression levels of key genes in the apoptotic cascade over the course of the infection, and consequences for metabolic energetics affecting honeybee performance.

Unreliability of Ribosomal genes of *Nosema ceranae* and *Nosema apis* for the quantification of spores and the parasitic charge.

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Two Microsporidia have been identified as honey bee parasites: *Nosema apis* and *Nosema ceranae*. Both infect the ventriculi of bees causing a disease known as Nosemosis. Up to now, some works have been published to quantify the spores or the parasitic charge in honey bee samples or tissues. The most employed genes to do this, use to be the Ribosomal genes since they are in more than one copy per genome. In this study, we studied

the *Nosema* spp. ribosomal genes in order to determine the reliability of these genes for quantification these parasites.

Primers annealing in different regions of ribosomal genes of both *Nosema* spp. were designed to get information of these gene regions. After performing the PCR analysis, cloning of PCR products and sequencing were done and then the sequences were compiled and analyzed for polymorphism.

Purified spore samples of *N. apis* and of *N. ceranae* were used to quantify the number of spores using some primer sequences available in the literature which used the ribosomal region as target for quantification. These samples were counted under phases contrast microscopy by four different experimented operators and the average was used as reference value. The results obtained were compared to those obtained after analysis using the RPB1 (RNA polymerase II) gene which is a gene of unique copy.

Some incongruent results were obtained among the samples analyzed and the primers used confirming some concerns related to the use of Ribosomal genes for quantification.

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European foulbrood (EFB) and American foulbrood (AFB) – Distribution, status and control & management strategies throughout Europe

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There are two significant bacterial diseases of honeybees; European foulbrood (EFB) caused by *Melissococcus plutonius* and American foulbrood (AFB) caused by the spore forming bacterium *Paenibacillus larvae*. In both diseases the bacteria multiply in developing honeybee larvae and, dependant on the dose and virulence of bacterial strain, infection may result in various levels of larval mortality. In EFB this is usually seen before capping of the brood and in AFB larval mortality usually occurs after capping. We conducted a survey based on a questionnaire, which was sent to each of the European countries. The questionnaire was designed to collect information on the distribution, legal status and control/management systems in place for EFB and AFB. The survey revealed a wide variation between legal status and national surveillance/control strategies throughout Europe. One of the most striking findings was the apparent lack of information on the distribution and prevalence of these two important diseases throughout many European countries.

Development of a probiotic mixture based on *Lactobacillus kunkeei*, to improve honeybee health.

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Native intestinal microbial community is essential to maintain an adequate nutrition, health and immunity against pathogens. The administration of probiotics as feed additives is a widely used strategy, both in humans and animals, in order to improve health.

In a previous work we obtained and characterized a collection of isolates from the midgut microbial community of healthy honey bees. Ten isolates were selected based on their biological characteristics (in vitro inhibition of *Paenibacillus larvae*, resistance to high sugar levels and low pH values, production of lactic acid, among others). The aim of this study was to evaluate those isolates as potential probiotics.

We assessed their *in vivo* pathogenicity on larvae and adult bees; their *in vivo* effect against *Paenibacillus larvae* and *Nosema ceranae*, and their ability to modulate the immune system of adult bees.

After oral administration of the bacterial preparations in sugar syrup, none of them resulted pathogenic for adult bees or larvae. Regarding their effect against pathogens, an increased viability was found in *P. larvae*-infected larvae, when a mixture of *Lactobacillus kunkeei* strains was added to their food. This mixture was also able to decrease the infection levels by *N. ceranae* spores. Finally, those isolates were able to modulate the expression of genes related to immunity.

These results suggest that *L. kunkeei* strains are promising candidates to be used in a probiotic preparation to improve honeybee health. Further assays are needed to assess their effect on colonies in the field.

Probiotic properties of honey bee gut microbiota isolated from Georgian honey bee samples.

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Recent research of insect microbial communities has shown crucial importance for host's normal physiology fulfilling particular functions such as production of antimicrobial metabolites, modulation of host immune response, possible involvement in carbohydrate breakdown, participation in bee bread maturation. However the composition of the microbial community is dramatically affected by multiple factors.

This work was aimed to investigate the microbial biodiversity and probiotic proper-

ties of fructophilic bacteria isolated from honey bee gut and bee bread collected from different regions of Georgia.

For isolation of targeted bacterial strains healthy honey bee colonies were selected. After collection and dissection of worker bees belonging to different ages, digestive tract were removed and subjected to microbiological investigation.

86 selected strains were typed by RAPD-PCR, species were identified by sequencing of 16S rRNA gene. 25 strains belonging to different RAPD cluster of each species were characterized. Adhesion properties, antibiotic susceptibilities, resistance to high concentration of sugars, antimicrobial activity against strains of honey bee pathogens such as *Paenibacillus larvae* and *Melissococcus plutonius* were studied taking into account importance of these properties for further selection of probiotic strains.

Putative beneficial capabilities of isolated bacterial strains studied in our work can serve as additional data for more deep understanding of host beneficial action and can be exploited as main criteria for selection of appropriate probiotic strains for agricultural applications. Different rate of antibiotic sensitivity observed within honey bee gut microbiota heightens antibiotic usage concern in beekeeping practice especially in developing countries.

Honey quality and self-medication against pathogenic honeybee bacteria in *Apis mellifera*.

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Bacterial pathogens take advantage of the homeostatic nest conditions in honeybee colonies. The most prominent agents are *Paenibacillus larvae* (American foulbrood, AFB) and bacteria associated with European foulbrood (EFB). Beside classical innate immune defence, honeybees use behavioural defence mechanisms to prevent or reduce pathogen infections. Foraging of antimicrobial plant compounds plays a key role for this social immunity. Secondary plant metabolites in nectar are well known for their antimicrobial effects. Yet these compounds are highly plant specific and the effects on bee health will strongly depend on the floral origin of the honey produced.

Here we test eight different AFB and EFB bacteria strains and the growth inhibitory activity of three different honey types with four different concentrations. Using a high-throughput microtiter plate based cell growth assay we show that all honeys have high antibacterial activity even in low concentrations when the antimicrobial effect of the sugar generated osmotic pressure was no longer effective. However, the two monofloral honeys (black locust, sunflower) appeared to be strain specific in its antibacterial activity. This effect did not correlate with any of the antibiotic physicochemical parameters of each honey. The specificity of the monofloral honeys, and the strong antimicrobial potential of the polyfloral honey suggests that qualitative variance in the honey stores of a colony may be highly adaptive for social immunity. Since worker bees not only feed themselves but also

larvae and other colony members, honey is a prime candidate acting as self-medication agent in honeybee colonies to prevent or decrease infections.

Recent advances in applied honey bee virus research: living between hope and fear.

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Over the past five years, the Ghent University has invested much in tool development for bee health screening. BeeDoctor is a PCR-based tool that allows rapid screening for multiple bee viruses and BeeClinic is a colorimetric DNA chip for detection of stress markers. The lab is now using these tools in different set-ups with national and international partners. Here we present research that was done in collaboration with the Flemish beekeeping sector. The studies focussed on virus infections and the outcome let us balance between hope and fear. Alarming was the finding of high virus loads of selected breeder queens and the risk of vertical transmission. The relationship between virus infection and offspring performance is now under investigation. Promising was the finding of an activated anti-viral machinery in a subset of the tested honey bee colonies. These are just a few of the numerous screenings that we recently performed with the in house developed tools.

Quantitative Virus Dynamics in Honeybee (*Apis mellifera* L.) Colonies along a New Expansion Front of the Parasite *Varroa destructor*

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Over the past fifty years, annual honeybee (*Apis mellifera*) colony losses have occurred, in parallel with the global spread of the honeybee parasite *Varroa destructor*. Indeed, *Varroa* mite infestations are considered to be a key explanatory factor for the widespread increase in annual honeybee colony mortality. The host-parasite relationship between honeybees and *Varroa* is complicated by the mite's close association with a range of honeybee viral pathogens. The 10-year history of the expanding front of *Varroa* infestation in New Zealand offered a unique opportunity to assess the dynamic quantitative and qualitative changes in honeybee viral landscapes in response to the arrival, spread and level of *Varroa* infestation. We studied the impact of *de novo* infestation of bee colonies by *Varroa* on the prevalence and titres of seven well-characterised honeybee viruses in both bees and mites, using a large-scale molecular ecology approach.

Our data reveal that the different viruses have unique quantitative dynamics in relation to *Varroa* infestation, resulting in a shifting succession of virus infections that ultimately leaves DWV as the predominant infection. Assumption-free analysis shows

consistent clustering of the data according to *Varroa*-infestation history, confirming a progressive change in the overall virus landscape co-incident with *Varroa* infestation. Both positive and negative associations were identified for several pairs of viruses, in response to the arrival of *Varroa*. These findings provide important new insights into the role of the parasitic mite *Varroa destructor* in influencing the viral landscape that affects honeybee colonies.

Resistance to Varroa: What have viruses got to do with it?

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Varroa destructor is currently the most devastating pest of honeybee colonies in large part because of its role as a highly efficient vector of several honeybee viruses, initiating and accelerating within-colony virus epidemics that kill the colony within a few years, unless the *Varroa* population is kept under control. Despite this lethal outlook, there are a few well-documented honeybee populations that have adapted to survive untreated *Varroa* infestations. One such population was established 15 years ago as a natural selection experiment on Gotland, an island in the Baltic Sea. Current research is focused on the different traits that this population has developed that permit it to survive long-term with untreated *Varroa* infestation. Here we will address the role that virus infections have played in the adaptation process and in the continuing survival of this population in the absence of *Varroa* control.

Russian honey bee molecular genetic study in the aspect of honey bee breed conservation and spread of RNA-containing virus infection

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Penetration of southern subspecies of honey bee *Apis mellifera* L. (*Apis mellifera carpatica*, *Apis m. carnica*) into populations of *Apis m. mellifera* L. due to uncontrolled honey bee breeding in the natural habitat of northern subspecies generates honey bee hybrid populations with reduced production quality, resistance to parasites and infections, and less viability in severe environment. Hybridization, thus, might contribute to the spread of infectious diseases and increasing threat of conservation of the pure *Apis m. mellifera* L. populations on the Russian territory. In the Russian honey bee breeds, we performed analysis of mtDNA variation and microsatellite variability in order to gain breed characteristics, which are necessary for pure breed conservation and hybrid reveal-

ing. Simultaneously, we detected the presence of RNA-containing viruses by RT-PCR. We detected RNA-containing viruses: in one bee colony, up to five or six different viruses were present. In populations of *Apis m. mellifera* L. (Tatarstan) and *Apis m. carpatica* (Adygea and Moscow region) and *Apis m. carnica* (Abkhazia), we revealed the presence of six RNA-containing viruses DWV, SBV, BQCV, KBV, APBV and CBPV. In honey bee colonies, DWV was spread with mean frequency 50% and SBV - 48%, other viruses were less frequent: APBV (16%), BQCV (13%), CBPV (12%) and KBV (9,6%). In honey bee families, double virus infection was detected in 33.9% and multiple infection - in 21.2%. In *Varroa destructor* mites, the presence of RNA-containing viruses (DWV, APBV) was also found by RT-PCR, thus, indicating the mites as virus transmitters. From Russian honey bee populations, sequences of RNA-containing viruses DWV (GQ422786, JX993278, KC138854, KC138852, KF274666), SBV (KC020673, KC020674, KF274667, KF274668) and BQCV (KC138853) were isolated, high level of identity with corresponding fragments of virus isolate sequences in Genbank was demonstrated (up to 85%).

Genetic diversity and virulence in the DWV quasi-species

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Deformed wing virus (DWV) is currently one of the most prevalent viruses in honey bees. It is the causative agent of the wing deformities, abdomen malformations and the reduction of the life span of highly infected bees. It is also strongly correlated with winter mortality of colonies.

Despite its importance as a threat to honey bee health, few studies have addressed the population dynamics and adaptive potential of DWV, particularly in relation to its pathogenicity (capacity to cause disease) and virulence (severity of disease). The adaptive potential of a virus is represented by the viral quasi-species: a cloud of multiple virus variants whose complexity provides the raw material for selection. This complexity can be accessed and quantified using Next Generation Sequencing (NGS) technology.

We used this to investigate the behavior and within-host genetic diversity of a pathogenic DWV quasi-species through fourteen successive passages of serial transmission to red-eye honeybee pupae (N=10 pupae per passage).

This process leads to the appearance of DWV symptom attenuation after only six passages. The DWV quasi-species of the different types of transmission progeny were analyzed with multiple techniques to address both the quantitative and qualitative aspects of the virus' contribution to virulence and symptom development. The host's and vector's contributions will be addressed in future analyses.

Honey bee queen health in correlation with virus transmission routes

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Honey bee colony losses have risen seriously in the past decade, especially in the USA. In Europe, average loss rates appear less dramatic and less frequent, but high losses are known to occur regionally. Scientists and beekeepers are struggling to find answers to this problem. Queen loss and infertility is frequently considered an important factor among the numerous possible reasons, and death of the queen outside the mating season invariably will lead to collapse of the colony, since long term colony survival depends on the queen producing a large number of offspring. Therefore, queen health is closely linked to colony health and survival. Viruses are amongst the honey bee pathogens known to have a negative impact on queen health. Here we present data demonstrating different transmission routes of viruses to the queen. While a direct impact of certain viruses to queen health remains to be shown, our findings provide insights to possible causes for queen loss and ways to avoid this risk.

Bullying females and other indicators for cryptic female choice in the Small Hive Beetle *Aethina tumida*

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The evolution of sexual conflict has produced a vast array of mating strategies in the animal kingdom. One commonly observed tendency, however, is for aggression to be associated with male competition for females. Most reports on female aggression describe competition for food and resources and very few reports of females competing for mates exist. In the few reports that do exist, this aggression tends to take place on a physiological level, for example through pheromones, as in social insect species. We describe the mating behaviour of *Aethina tumida* as well as the active involvement of females in it. It is primarily the females who show aggressive behavior towards other beetles and fiercely interrupt matings. During mating behavior males remain on the backs of females for a long time and show reduced precopulatory courtship, although they may display courtship behavior during copulation. All of these features indicate cryptic female choice mechanisms in the small hive beetle. We further address the importance of aggregations for successful reproduction as well as our findings on homosexual copulation. All in all, females seem to be the dominant sex in this mating system, which is perfectly adapted to overcome the honey bee host through mass reproduction (*Apis mellifera* sp.).

The next threat to European apiculture? Effects of the ectoparasitic mite *Tropilaelaps mercedesae* on workers of western honey bees, *Apis mellifera*

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The ectoparasitic mite *Tropilaelaps mercedesae* has switched hosts from the giant honey bee *Apis dorsata* to the western honey bee, *Apis mellifera*, in Asia. Similar to *Varroa destructor*, *T. mercedesae* is also able to successfully reproduce in worker brood of its new host *A. mellifera*. However, the effects of *T. mercedesae* on *A. mellifera* are poorly understood. Here, we artificially infested developing *A. mellifera* workers (N=40) immediately post-cell capping to investigate the influence of *T. mercedesae* on host morphology and longevity in the laboratory. We observed that infested workers had significantly lower emergence mass, higher proportion of deformed wings, and reduced adult lifespan. These results underline the lethal impact of *T. mercedesae* on *A. mellifera*, and therefore further support the need for research to better understand and mitigate its effects on *A. mellifera*, as well to prevent introduction of this mite to novel, susceptible host populations, particularly in Europe and North America.

Activities of European network of National Reference Laboratories during the year 2011 in the field of honey-bee health

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The Anses Sophia-Antipolis laboratory was named EU Reference Laboratory (EU RL) for honey-bee health in 2011. One of the first tasks of the EU RL was to inventory the analytical capacities of the National Reference Laboratories (NRLs) in the European Member States (MSs) regarding honey-bee disease diagnosis. A detailed questionnaire was sent to each MS, in addition to Norway and Switzerland. The questionnaire aimed at gathering information on the analytical capacities of the MSs in 2011. The enquiry targeted all honey-bee diseases and pathogens, from notifiable diseases, as the American foulbrood (AFB), *Tropilaelaps* spp. and the Small hive beetle, to pathogens frequently observed in the colonies, as viruses. Questions focused on the number of analyses realised by the NRLs during the year, the type of methods used, the validation and accreditation of the methods and, finally, the organisation of and the participation to inter laboratory proficiency tests (ILPTs). Twenty-seven NRLs answered the questionnaire. Results showed a great activity in Europe regarding honey-bee health seeing as more than 300,000 analyses were carried out. In 2011, 100% of the NRLs were in capacity to diagnose (AFB), and more than 90% to diagnose European foulbrood (EFB), nose-mosis and varroosis. Almost 60 methods were accredited and some methods were in an accreditation process. More than

30 new methods were set up in 10 NRLs, showing the dynamic improvement of the laboratories. Methods used included various techniques as macroscopic analyses, microscopy or molecular biology. Moreover, nine ILPTs were organised on AFB, EFB, noseiosis or varroosis. This overall enquiry is the first one conducted for the European NRLs involved in honey-bee health and allowed to highlight the strengths and the areas of improvement to follow to better diagnosing and then better protecting honey bee.

Contingency plan for exotic pests and diseases of honeybees.

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The overall purpose of the contingency plan is to protect the honeybees in Sweden from exotic diseases, parasites and pests. The plan is primarily designed to ensure quick action at the discovery of the small hive beetle, the tropilaelaps- and tracheal mite and the Asian hornet *Vespa velutina*. The plan can also be used as an action plan for findings of other exotic diseases, parasites and pests not mentioned in this document.

The contingency plan shall:

- Lead to minimize and control an outbreak, so that the harmful effects are as small as possible.
- Help to create effective biosecurity at all levels to minimize the risk of introduction of diseases, parasites, pests and invasive species.
- Be regarded as a model description for an action plan for the possible detection of any exotic disease, parasites or pests in Sweden in the future.

The contingency plan is not only directed to the authorities, but should also provide assistance to beekeepers in the form of knowledge about detection and control of diseases, parasites and pests.

The plan identifies the different roles and responsibilities of the authority, the beekeepers, their organizations and other stakeholders towards achieving the stated objectives. The plan sets the organizational structure with responsibilities and legislation needed to strategically and tactically handle an outbreak. These measures are based on the knowledge we have today from experts' advice on the control methods for the diseases, pests and predators. These measures are designed to minimize the damaging effects on beekeeping and other related activities.

EPILOBEE : Results from a pan-European epidemiological study on honeybee colony losses 2012-2013

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For the first time, an active epidemiological surveillance programme on honeybee colony (*Apis mellifera* L.) mortality (EPILOBEE) has been implemented in Europe. Each member state has developed a surveillance protocol based on guidelines produced by the EURL to harmonize the surveillance procedures. Three visits were performed by bee inspectors: before winter 2012, after winter and during the beekeeping season. Farming practices and clinical manifestations of the main infectious and parasitic diseases were recorded through a detailed questionnaire resulting in the collection of a tremendous amount of data. Between autumn 2012 and summer 2013, 31 832 colonies located in 3 284 apiaries were fully visited by 1 354 bee inspectors in 17 member states (total of 8 572 visits of apiaries).

The production of colony mortality rates and disease prevalence in all the member states demonstrated that the methodology was fully implementable. Winter colony mortality rates will be presented in details. Prevalence of AFB, EFB, varroosis and noseosis based on clinical signs observed by bee inspectors will be described. This programme generated a remarkable work in the member states taking part to EPILOBEE, involving stakeholders from the ministry to the field. The EU-RL for honeybee health wishes to thank all participants for their substantial involvement.

EPILOBEE project resulted in the compilation of a tremendous amount of data, enabling the filling of a web based database (103 930 laboratory analyses were recorded in March 2014). Future data analyses will unquestionably explore the statistical links between the colony mortality and some risk factors.

Honey bee health screening reveals a broad set of pathogens, from which *Crithidia mellificae* correlated with winter losses

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Since the last decade, unusually high honey bee colony losses have been reported mainly in North-America and Europe. Here, we report on a comprehensive bee pathogen screening in Belgium covering 363 bee colonies that were screened for 18 known disease-causing pathogens and correlate their incidence in summer with subsequent winter mortality. Our analyses demonstrate that, in addition to *Varroa destructor*, the pres-

ence of the trypanosomatid parasite *Crithidia mellificae* and the microsporidian parasite *Nosema ceranae* in summer are also predictive markers of winter mortality, with a negative synergy being observed between the two in terms of their effects on colony mortality. Furthermore, we provide evidence for a parasitizing phorid fly, identified a new strain of Lake Sinai Virus (LSV), and confirm the presence of other little reported pathogens such as *Apicystis bombi*, Aphid Lethal Paralysis Virus (ALPV) strain Brookings, *Spiroplasma apis*, *Spiroplasma melliferum* and *Varroa destructor* Macula-like Virus (VdMLV). Finally, we could demonstrate for the first time that ALPV and VdMLV replicate in honey bees. We also noticed a significant correlation between the number of pathogen species and colony losses. Overall, our results suggest that we have to look a broader set of pathogens to understand the 'driver' pathogens, one of the likely causes of the current honey bee decline in Europe.

Sanitary survey of honeybees on Reunion island in a varroa and CCD-free context

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Apiculture is a major activity on Reunion, a small island in the south west of the Indian Ocean. 272 beekeepers and 13,000 colonies were officially registered in 2012 however with an overall estimated population of about 500 beekeepers and 30,000 colonies. No major sanitary problem had ever been recorded on the island neither any study had been conducted on honeybee health. So the aim of this study was to survey honeybees (*Apis mellifera unicolor*) health on the island.

The survey was conducted on 115 healthy colonies randomly sampled from April to October 2012 from 60 apiaries of 39 beekeepers. For each colony, 30 honeybee workers, 200 inner honeybees and 20 larvae were sampled. Several pathogens and parasites were investigated: 3 ectoparasites (*Varroa destructor*, *Tropilaelaps* sp. and *Acarapis* sp.), 2 bacteria (*Melissococcus plutonius* and *Paenibacillus larvae*), two nosema species (*Nosema apis* and *N. ceranae*) and 7 viruses (DWV, ABPV, CBPV, BQCV, DWV, KBV and SBV). During this survey CCD was investigated (on visual observation and reporting).

Colonies are *V. destructor*, *Tropilaelaps* sp. and *P. larvae* free. *Acarapis* prevalence was estimated at about 16%. *M. plutonius* was found in 23% of the colonies. All the samples were positive with *N. ceranae*, only one case of *N. apis* was detected. For viruses, BQCV, CBPV and ABPV were detected. .

Despite the presence of several pathogens (viruses such as Nosema), no CCD had been reported in the last two years. All these results are discussed in the light of a tropical bee sanitary context without *V. destructor*.

Occurrence and distribution of deformed wing virus, acute bee paralysis virus, sacbrood virus and chronic bee paralysis virus in honey bees (*Apis mellifera*) in Serbia – a Real-time RT-PCR based survey

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The honey bee *Apis mellifera* L. is susceptible to innumerable pathogens including viruses, bacteria, protozoa and parasitic mites. Diseases caused by certain honey bee viruses may possibly lead to serious epidemiological and economic consequences in infected apiaries. The occurrence and distribution of deformed wing virus (DWV), acute bee paralysis virus (ABPV), sacbrood virus (SBV) and chronic bee paralysis virus (CBPV) was investigated in *Apis mellifera* samples from different regions in Serbia using Real-time RT-PCR. The most prevalent virus, DWV, was present in 82% of adult bee samples, followed by ABPV in 50%, SBV in 34% and CBPV found in 14% of samples. Simultaneous infection with up to three viruses was identified in 64% of samples, with DWV-ABPV and DWV-SBV-ABPV infections being the most frequent (20%). Single viral infection was found in only 30% of the samples. Samples negative for all four viruses comprised 6% of investigated colonies. The Real-time RT-PCR assay with the use of probe based chemistry (TaqMan) proved to be more sensitive than conventional RT-PCR in the test encompassing serial dilutions of the positive samples. The highest efficiency of the reaction was obtained with a concentration of TaqMan probe of 400 nM, primer concentration of 800 nM and 2 ng of RNA template in a 25 µL reaction volume. This paper describes the detection of honey bee viruses in Serbia using Real-time RT-PCR technology. The results indicate the presence of monitored viruses in almost whole territory of Serbia.

The “BEE MONITOR” web application as practical tool to manage multifocal clinical trials on honeybees

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The management of the data obtained from clinical trials on honeybees is a practical need to compare and to evaluate the efficacy and the toxicity of experimental veterinary medicines in different apiaries. This necessity can be given for example by mega-projects realized between Countries to consider the performances of the same active principle on the honeybees in different ecosystems and climates. The Specialist Service of Honey Bee Production and Pathology of Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana (IZSLT) developed a modular and flexible web application in order to receive live all the informations by multifocal field trials. Through an authentication module, the

web application permits to submit to the project coordinator the data collected directly on field with a mobile-friendly interface or to contact the referent researcher of the field trial and receive real-time updates on the protocol. The reference person (administrator) can georeference the apiary, assign to it to a beekeeper and monitor the activities realized. An automated email notification system informs the beekeeper of the trial steps and the daily activities. Online fields can be adapted to different activities and the beekeeper can print a QR-code for each hive that allow to directly access and update its data into the web application. All records, arranged in a database, can be shared between all researchers and further implementations of the application will consider the integration of statistical analysis. This software can be freely tested and implemented by other researchers to meet their needs.

Dissecting the hygienic behavior in *Apis mellifera* (Hymenoptera: Apidae).

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The hygienic behavior is described as the ability of the honey bee *Apis mellifera* (Hymenoptera: Apidae) to detect, uncap and remove diseased brood from the hive. This behavior is performed by middle-aged workers and has developed as an important form of social immunity in the colony. Previous studies showed that individual bees that express the hygienic behavior (hygienic bees) have a higher olfactory sensitivity and responsiveness than non-hygienic bees and this variability could be explained by a genetic propensity to engage the hygienic tasks. The aim of the present study was to explore the genetic basis of this trait in honey bees by analyzing the gene expression profiles of candidate genes involved in olfaction, learning and social behavior. We studied 4 candidate genes (*octopamine receptor Oa1*, *smell impaired*, *rodgi* and *pheromone-binding protein*). Total RNA and cDNA were obtained from the head of middle-aged hygienic and non-hygienic workers. Gene-expression profiles from each group of bees were obtained using real time quantitative PCR (RT-qPCR) targeting transcripts of each candidate gene, using the *actin* and *Rp49* as reference genes. Hygienic bees tended to show higher *Oa1* transcript levels than non-hygienic bees. The other candidate genes showed no change between these groups of bees. Together with the analysis of other candidate genes, already in progress, we will considerate the underlying molecular mechanisms involved in signal transduction, communication, neurophysiology associated to hygienic behavior and providing newly information for marker-assisted selection for this commercially significant trait.

The diversity and ecology of the bee-loving fungi, *Ascosphaera* and *Bettsia*, in Europe.

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Ascosphaera and *Bettsia* are bee-specialist fungi that complete their entire life cycle within the nests of bees. Both genera are found world-wide wherever bees are present. Approximately half of the species of *Ascosphaera* are pathogens of bee larvae causing the disease commonly known as chalk brood while the remaining species are either saprotrophs or their potential as bee pathogens has not been verified. Beyond species diversity, very little is known about the ecology and distribution of *Bettsia* and the majority of *Ascosphaera* species. We review what we currently know about the ecology and evolutionary relationships of these bee-loving fungi, their diversity in Europe, how this knowledge is relevant to honey bees, and what future directions we should take to begin to fully understand the role of these fungi as pathogens or beneficials in the bee habitat.

Population Genetics and Conservation

Organizers: Alice Pinto, Per Kryger

Population genetics is a field of major importance to the conservation of bee diversity. In relation to the honey bee *Apis mellifera*, in the past, breeders mostly focused on *A. m. ligustica* and *A. m. carnica*, and in recent decades also on hybrid heterosis effects as in the Buckfast honey bee. However, there are still rather pure subspecies in isolated areas of Europe, which deserve attention and protection.

The symposium aims to present new results concerning the description of bee genetic diversity, development of new methods and initiatives for the conservation of locally adapted populations in its wider sense including not only honey bees but also bumble bees and solitary bees.

Invited speakers: Maria Bouga, Cecilia Costa, Raffaele Dall’Olio, Marina Meixner, Irene Muñoz

What do whole genome sequences tell us about the population history of honeybees?

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We performed whole genome sequencing of 140 samples of *Apis mellifera* collected from across the globe to comprehensively characterize patterns of variation across the genome, resulting in the identification of 8.3M SNPs. We applied coalescent approaches to illuminate the population history of honeybees and the processes underlying the patterns of diversity. A major finding of this study is that a substantial proportion of variation is shared between all continental groups. Levels of variation were surprisingly high in all populations we sampled. These values correspond to population sizes of several orders of magnitude larger than previously estimated from local variation at drone congregation areas. Domestic and Africanized lineages appear as diverse as their ancestral populations, indicating a lack of domestication or founder bottlenecks. In general, loss of genetic variation does currently not appear to be a major threat to honeybees. However, we uncover evidence that honeybee population sizes have varied greatly over evolutionary time. We demonstrate that linkage disequilibrium decays extremely rapidly in bees and we infer extremely high recombination rates across the genome, consistent with rates measured in experimental crosses. We used patterns of variation to investigate population history using a genealogical concordance method. We estimate that major continental groups diverged from each other ~200,000 years ago, but that current subspecies with similar ancestry are much younger, only 20,000-30,000 years old.

The gene geographic analysis of Burzayan population of *Apis mellifera mellifera* L.

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High honeybee colony losses in North America and Europe again recalled the importance of preservation of one's gene pool (Johnson, 2010). The 43 Apimondia Congress (2013) have noted the research of local aborigine populations as a key direction of modern conservation beekeeping. Aborigine honeybee populations are being restored in Sicily (Costa, 2013), Greece, Cyprus (Bouga, Hatjina, 2013) and other parts of Europe. Since 2008 the gene geographic analysis of Burzayan population of *A.m. mellifera* in the South Urals was performed by mitochondrial (tRNA-Leu - COII) and 9 microsatellite (Ap243, 4A110, A24, A8, A43, A113, A88, Ap049 и A28) markers. The population area, genetic structure and molecular diversity have been analyzed in this study. In total samples from 1102 colonies, 206 apiaries and 10 districts in a mountain-wood zone were analyzed. The sequence of the locus COI-COII mtDNA helped identify new M line mitotypes, which can be used as genetic markers for Ural honeybee. The population border was mapped, one

is occupying an area two times greater than previously thought. The total number of genetically pure colonies reaches nearly 10 thousand. The genetic differentiation of Burzyan population from other *A.m. mellifera* in the Urals was shown with the optimal conservation strategy for the honeybee in Russian conditions being proposed.

Reintroduction of the endangered *A. m. siciliana* in Sicily: genetic and morphometric tools to support management of mating stations and conservation islands.

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Efforts for *A. m. siciliana* conservation began in the late '80s, when colonies were isolated on the small Ustica island to avoid introgression with the Italian yellow bee *A. m. ligustica*. A single queen breeder and CRA-API (*Apiculture and Sericulture Unit* of the Agricultural Research Council) cooperated until the present days to preserve a pure population on small conservation islands.

The three year reintroduction project APESLOW: "Reintroduction and conservation of endangered subspecies *Apis mellifera siciliana*, Dalla Torre 1896: a tribute to Prof. Genduso", has come to its final year: here we present a brief overview of the project and the results of the first two years of monitoring on *A. m. siciliana* populations. About 400 honeybee colonies have been investigated at mitochondrial and nuclear level, results on about 150 samples were confirmed with morphometrical methods, in order to maximize the reintroduction effort on Sicily main island, to achieve an efficient management of main island mating stations and to implement original conservation islands' population with additional genetic variability.

Preserving local bee population and beekeeping heritage in a French national park

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The Cevennes National Park is a protected area that has been recently recognised for its exceptional biological and cultural heritage by UNESCO. Cevennes region is renowned for centuries as a 'land of honey' where beekeeping used to be characterized by a traditional beehive made out of hollow chestnut-trunks. Log-hive apiaries constitute an extremely old beekeeping practice, and were originally populated by local black bees (*Apis mellifera mellifera*). Nowadays, global change, influences practices and most beekeepers have adopted framehives. Changes have also led to decrement of the local bee subspecies and to its genetic homogenization. In order to preserve its natural and cultural heritage,

the Cevennes national Park launched an interdisciplinary study to record this disappearing traditional ecological knowledge. A major goal of the study is to better understand the distribution of black bees through comparative sampling of bees in framehives, in log-hives and in the wild. Identification of bees is performed by both morphometrical geometry and mitochondrial DNA analyses (COI-COII). Morphometrical results establish that black bees are still present throughout the national Park, but hybridization is also ubiquitous and affects more than 30% of sampled bees. mtDNA sequencing shows that west European evolutionary lineages (M) have almost disappeared in local framehives but still persist in some log-hives. Collected information will be used by the national Park as a means to implement a conservation policy. The challenge is to bring together all beekeeping stakeholders and their respective expectations in order to achieve the conservation of this subspecies.

Population structure of Iberian honey bees: concordance among mtDNA, geometric morphometrics and SNPs

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No other honey bee subspecies has caught so much attention from evolutionary biologists as *Apis mellifera iberiensis*. For over 25 years, Iberian honey bee populations have been surveyed using a wide range of markers, including morphometry, allozymes, mtDNA, microsatellites, and more recently SNPs. Despite the numerous studies, diversity patterns of Iberian honey bees are still not fully dissected and understood. In this study we performed a fine-scale sampling at both geographical and genomic level. Samples from over 711 colonies were collected across three north-south transects. These samples were sequenced for the mitochondrial tRNA^{leu}-cox2 intergenic region, scored using geometric morphometrics of forewings, and genotyped for over 383 SNP loci. The three sets of markers concurrently supported the existence of a north-south cline, a pattern that contrasts with the absence of structure inferred from microsatellites. To test the hypothesis that selection has shaped and maintained the nuclear cline, we employed four FST-based outlier methods on the SNP data set. These analyses enabled detection of 74 outlier loci, which supports the selection hypothesis. However, neutral structure, inferred following removal of outliers, was able to recapture the north-south cline, which suggests that selection is not the only evolutionary force shaping the Iberian cline. Our findings further highlight the complexity of the Iberian honey bee diversity patterns and reinforce the importance of this southernmost European territory as a reservoir of *Apis mellifera* genetic diversity, a resource increasingly important in a rapidly changing and demanding world.

Within-colony genetic diversity of honey bees (*Apis mellifera* L.) in relation to *Nosema ceranae* infection.

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Within-colony genetic diversity has been proved to be clearly important for honey bee (*Apis mellifera* L.) fitness. Multiple mating by honey bee queens results in increased within-colony genetic diversity, which has been shown to confer numerous benefits for colony health and productivity. Selection for increased intracolony disease resistance has been proposed as a hypothesis for the origin of polyandry, as queens multiple mating produces genetically diverse workers. Within-colony genetic diversity thus implicates differences in susceptibility to a determined disease, which increases the probability of the colony, as a whole, to survive. Here we present the results of a study that aimed to test this pathogen hypothesis for polyandry in the case of the parasitism by the microsporidium *Nosema ceranae*. In this study we have analyzed the level of genetic diversity (using microsatellite markers) of six different colonies from the same apiary. Two of them collapsed after three years of infection by *N. ceranae*, while the four remaining colonies show marked differences in their responses to the *N. ceranae* infection. The results showed no differences among them and are discussed in relation to the parasite hypothesis stating that the increase of the genetic diversity contributes to colony resistance by reducing the probability of colony collapse that might occur if a colony was genetically homogeneous.

Selection on Varroa sensitive hygiene (VSH) in commercial European Carnica bees

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During 1991 – 1993, we firstly investigated the removal of Varroa infested brood cells in 144 Carnica colonies of different breeding lines and found 0 to about 50 % of the single infested cells removed within 7 to 10 days. Significant effects of the genetic origin showed the inheritance of the trait and qualified it as a relevant character for selection on mite resistance.

Different methodical approaches were tested in order to establish a simple and standardized method for the estimation of brood hygiene behavior in large scale performance testing. With regard to a reliable repeatability of test results and a significant correlation to the removal of Varroa infested brood a pin test assay was finally established. It became part of the breeding program of the AGT breeder association (www.toleranzzucht.de) with several thousand colonies tested each year. The results are involved in the central estimation of breeding values for Varroa resistance (www.beebreed.eu).

Harbo & Harris (2005) achieved a strong expression of VSH by selecting colonies for a high level of nonreproducing Varroa mites (SMR). Since those bees proved to be highly

resistant under field conditions, we recently started to test for SMR in our preselected Carnica population. From 26 colonies checked in summer 2013, 26,9 % showed increased levels of SMR with up to 42 % of nonreproducing mites in worker brood. We found a weak but positive correlation between SMR and pin test results ($r=0,25$, $p=0,224$) and a negative correlation with the mite infestation of the colonies ($r=-0,47$, $p=0,016$).

Allozyme and microsatellite DNA polymorphism and markers, usable for discrimination of local Bulgarian honey bees.

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The genetic variability of honey bee populations from 24 Bulgarian provinces and populations of *A. m. carnica*, *A. m. caucasica*, *A. m. ligustica* and *A. m. macedonica* has been studied using allozymic analysis of six enzymic systems (MDH, ME, EST, ALP, PGM and HK) corresponding to 6 loci and DNA analysis of 9 microsatellite loci (Ac011; A024; A043; A088; Ap226; Ap238; Ap243; Ap249 and Ap256). All of the studied allozymic loci were found to be polymorphic in most of the populations. Four alleles were detected at Mdh-1, Me and Pgm loci and three alleles – at Alp and Hk loci. Est-3 locus was polymorphic with six alleles. All of analyzed nine microsatellite loci were found to be polymorphic in all of the populations with the presence of a total 121 alleles. Thirty five private alleles were observed in 19 of the studied populations. Nei's genetic distance between studied populations was calculated and UPGMA and neighbor-joining phylogenetic trees were constructed. Genetic markers, usable for discrimination of Bulgarian honey bees were described in this study. Some differences between Bulgarian and other *A. m. macedonica* honey bee populations were discussed which suggests that local Bulgarian honey bee could be a different ecotype of *A. m. macedonica*. The data of this research provide new information concerning the genetic variability and differentiation among Bulgarian honey bee populations and would be useful for selection and conservation purposes.

Survival of feral honey bee colonies in Poland

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Honey bees (*Apis mellifera*) are native in Europe except its most northern part. In natural conditions they inhabited hollow trees. In recent centuries the bees were partly domesticated and currently most colonies are kept by beekeepers in hives. The feral colonies are still present in some areas of Europe but their density is relatively low. The most impor-

tant factors which affect density of the population are degradation of natural environment and presence of human introduced pathogens. In particular it is not clear if the untreated feral colonies are able to tolerate *Varroa destructor* which was introduced few decades ago and now is present in almost all colonies in Europe.

We have investigated survival of the feral colonies living in tree hollows in Poland. The colonies were repeatedly sampled and genetic data of the bees were analysed. This allowed to determine if the same maternal line is present at particular site. Moreover, the feral colonies were compared with managed colonies from nearby apiaries to determine whether bees living under natural condition show bias towards the native subspecies *A. m. mellifera*.

The feral and managed colonies did not differ significantly in terms of their assignment probabilities. We have found out that mortality of the feral colonies was relatively high. Many of the feral colonies were destroyed by people, either by cutting trees, or removing colonies. However, some of the colonies were able to survive more than three years. This suggests that the feral colonies can at least partially tolerate *Varroa* mites.

Genetic diversity of *Apis mellifera* populations in the Mascarene Archipelago in the Indian Ocean

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The islands of the South West of the Indian Ocean are a hotspot of biodiversity with high rates of endemism. Honeybees play a major role in these insular tropical ecosystems, interacting with indigenous and endemic species but also with exotic and invasive ones. In Madagascar (heart of the hotspot of biodiversity) the honeybee has already been described as *Apis mellifera unicolor*, an endemic subspecies belonging to the African lineage. However, knowledge about the established honeybee populations from other islands is extremely limited and their origins and diversity remain unknown. So, we performed a study aiming to 1) characterize the evolutionary lineages and subspecies and 2) determine the genetic diversity within honeybee populations of the area. A broad sampling from La Réunion (n = 2112 colonies), Mauritius (n = 355) and Rodrigues (n = 524) was performed. Maternal origin of *A. mellifera* individuals was assessed with molecular tools targeting the mitochondrial tRNA^{Leu}-COII intergenic region (*Dra* I test and sequencing). Then, nuclear genetic diversity was investigated with 15 microsatellites markers on all samples. Mitochondrial analyses revealed striking differences among islands. On La Réunion, 91% of the sampled colonies were attributed to *A. mellifera unicolor* and 4% to European lineages; in Mauritius the proportion of African and European lineages were equivalent (45% and 43%, respectively) whereas Rodrigues was exclusively represented by European C lineage subspecies. Nuclear analyses showed i) high genetic diversity comparable to continental populations and ii) that hybridization occurred between subspecies in all islands but at different levels.

The genetic diversity of *Apis mellifera* L. in western Africa

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To improve our knowledge on the genetic variability of *Apis mellifera* in western Africa, 148 colonies at 39 sites were sampled throughout Nigeria and parts of Niger, Cameroun and Chad, covering altitudes between sea level and 2000 m. The samples were subjected to morphometric analysis, analysis of mitochondrial DNA (tRNA^{Leu}-COII intergenic region and sequence variation of the CytB gen), and are currently analysed for microsatellite variation. Results of the morphometric analysis place the samples in the vicinity of *A. m. adansonii* and *A. m. jemenitica*. Restriction analysis of the intergenic region with *DraI* identified four known mitochondrial haplotypes: A1 (n=62), A4 (n=70), A4' (n=15) and A14 (n=1). We observed a conspicuous pattern in the geographic distribution of haplotypes, with a high prevalence of A4 and its variant A4' in the northwest, and A1 dominating in the southern and northeastern parts of the study area. As there is no obvious correlation with patterns of precipitation and humidity, this haplotype distribution may be the result of a historical bottleneck event; however, alternative hypotheses are being explored to explain the apparent lack of variation. A phylogenetic analysis based on the cytB sequence data together with reference data placed most samples into the sub-Saharan branch of the A-lineage, but a few samples also cluster with *A. mellifera intermissa* reference data from north Africa.

The combined results will be discussed and are expected to contribute to our understanding of population structure and honey bee biodiversity in Africa.

The influence of genetic origin and environment on the survival and performance of European colonies

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The aim of this study was to investigate the effects of genotype-environment interactions on the survival, performance and disease susceptibility of honey bee colonies headed by queens originating from several areas in Europe. We included 16 different genotypes from different backgrounds (some from breeding programs with strong focus on specific traits, others from conservation programs with little selection) which were located in 21 locations in 11 countries across Europe. In each location the local strain was tested together with at least 2 other strains of different origin. The experiment ran from October 2009

until March 2012. No chemical treatments for Varroa were enacted.

Of the observed colonies, 16% survived until the end of the experiment. A survival analysis showed highly significant effects of the test location, the genotype and the origin of queen (local vs. non-local) on the longevity of colonies. Colonies of local origin survived on average 80 days more than colonies of non-local origin. Other measured traits showed high variability among genotypes and locations. Diseases were highly influenced by environment. Some traits of colony development and behavior showed specific adaptation to environment.

Overall, our experiment showed that there is a high diversity of bee populations in Europe, which can ensure a high genetic adaptability of European honey bee populations. To enact conservation of this diversity, local breeding activities should be promoted and encouraged throughout the native range of *Apis mellifera*.

Conservation of local adapted honey bees: options and obstacles

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Preservation of genetic diversity is connected with the preservation of characters that favour adaptation of honey bees to their local conditions. But conservation of local adapted honey bees, demands for the active involvement of beekeepers' and, in general, local or national agreement. Current experience from several attempts to facilitate conservation strategies, have demonstrated that protection of honey bee populations is possible, but difficulties have been also arisen.

A Certification System has been established in Greece concerning honey bees' queen genetic origin and quality. The main reasons to establish such a system, was the wish to control the trade of local honey bee queens. The certification of origin and quality is an essential step in this process, and it can contribute in preserving the local honey bee diversity. The entire beekeeping community didn't accept this kind of control and finally only a few queen breeders have been involved in the system.

A similar experience comes from the Amorgos Island (Aegian Sea), where there is demand for local bees, in order to conserve these for future generations, collide with commercial interest of some beekeepers and the local community.

Local agreement and collaboration with the beekeepers are necessary requirement for any kind of attempt for preservation of local adapted honey bees.

Bees and cereal farming systems: from ecological studies to developing innovative bee-friendly cropping systems

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Supporting the protection of pollination services has both ecological and economic concerns and needs an alternative management of the farming landscape, taking into account the protection of bees as a priority.

The POLINOV project has 3 goals: i) a better knowledge of the current agricultural landscape's impact on the ecology of wild or honeybees, thanks to the monitoring "Ecobee" facility in an intensive cereal farming system of western France (Rollin et al., 2013; Odoux et al., 2014), ii) new farming system models which respect preservation issues for bees, iii) Assess the performance and sustainability of these systems from the point of view of farmers and beekeepers using the multi-criteria analytical tool (DEXi).

We showed that the efficient conservation of wild flora for wild bees depends on its connection with the cropping systems and the type of crops; and that the honeybees' populations largely forage on wild flora between the flowering of rapeseed and sunflower (which provide main honey harvests in beekeeping). Therefore, the agro-environmental scheme for the bees should be carried out according to the spatial landscape composition. Innovative measures were put forth to regulate food resources both in quantity and quality, thus benefiting the bee population and apicultural production. Innovative techniques have combined the reduced use of insecticides, appropriate management of the land and the introduction of cover crops producing nectar and/or pollen. Plant successions with low pesticide treatment and producing nectar or pollen should be encouraged.

Our work on the ecology of different bee species takes into account both the technical and economic issues relating to bees' conservation, beekeeping and farming. The most successful innovations for bees have a social and economic cost for the farmer that he is not yet ready to meet. In fact the optimisation of a pollination service in an area requires strong support from both agricultural stakeholders, and non-agricultural actors.

This work was supported by grants from the French Ministry of Agriculture (CAS-DAR, POLINOV program n°9535).

Outbreeding on Drone Congregation Areas of the giant honeybee (*Apis dorsata*)

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The giant honeybee *Apis dorsata* is native to South-East Asian forests where it establishes single comb nests in the open under tree branches, rocks or human-made structures. Often colonies form dense aggregations which can include up to 200 often closely related nests in the same location, setting the stage for inbred matings. Yet, like in all other *Apis* species, *A. dorsata* queens mate on the wing on drone congregation areas (DCAs) where thousands of males group in order to reproduce. We here study if the drone composition on DCAs facilitates outbreeding including both spatial (three DCAs) and temporal (subsequent sampling days) dynamics. We compare the drones' genotypes at ten microsatellite DNA markers with those of the queen of seven colonies located close to the DCAs (Tenom, Borneo, Malaysia). None of 430 sampled drones originated from any of these nearby colonies. Moreover, the composition of the DCAs was highly dynamic over time. Whereas sampling day had a significant effect on the drone composition, DCA location and the subpopulation of origin did not. Hence different colonies contribute to the three DCAs on different days, but the three DCAs did not differ in drone composition with respect to the drone contributing colonies on that specific day. Our results show that the flight behaviour of *A. dorsata* drones prevents matings with potentially related queens from the aggregation. In particular, the temporal dynamics of the drone composition maximizes the genetic variance among the mating partners of those queens performing multiple mating flights on subsequent days.

Genetic differentiation of *Varroa destructor* after its host switch from *Apis cerana* to *Apis mellifera*

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When the Western honeybees *Apis mellifera* was introduced to South-East Asia, it came in contact with a great diversity of pathogens and parasites. Among them, the *Varroa destructor* mite successfully switched from its native host *Apis cerana* to the introduced species. Following this event, the mite has spread in most regions where *A. mellifera* occurs and caused tremendous damages to the Western honeybees. However, very few studies have focused on the situation in South-East Asia where both its original and new hosts are commonly found. In this study, we investigated the genetic differentiation of *V. destructor* in North Vietnam between mites coming from both *A. cerana* and *A. mellifera*. We first sequenced the mitochondrial Cytochrome Oxidase I gene of mites coming from both hosts to estimate their haplotype. We then genotyped the mites at six microsatellite DNA markers to infer the patterns of dispersal between its two hosts. Our results indicate that

no transfers are currently occurring between mites coming from the two hosts in Northern Vietnam. Furthermore, the genetic diversity of the mite within its new host is very limited as host switch has only occurred in rare cases and the mite reproduces quasi-clonally. To further identify traces of selection within *V. destructor* genome involved in host switching, we sequenced the whole genome of mites coming from both hosts and compared it.

Evaluating the performance of a variable number of SNPs for genetic identification and introgression analysis in the dark honey bee

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Genetic identification and introgression analysis using molecular markers is an important tool for management and conservation of honey bee subspecies. High density assays featuring Single Nucleotide Polymorphism (SNP) markers can be exploited to create a reduced panel containing the most informative markers for these purposes. The objective of this study was to determine the minimum number of SNP loci required to verify the origin of dark honey bee individuals and to provide accurate estimates of the level of C-lineage introgression into their genome. We estimated allele frequencies of 1183 SNPs from 113 drone honey bee individuals sampled in the natural ranges of *A. m. mellifera*, *A. m. carnica* and *A. m. ligustica*, and evaluated the discriminant power of the SNPs using a variety of metrics and approaches including the Weir & Cockerham's FST, Delta, informativeness (In), PCA and an FST-based outlier test. Taking into account the less expensive multiplex assays made available by Illumina®, we created 5 panels of 48-, 96-, 144-, 192- and 384-SNPs with the average top-ranked loci and tested them to obtain the probability of assigning individuals to the correct origin and to calculate the admixture level using each panel. The analyses showed no significant differences in the introgression proportions produced by the different SNP panels, suggesting that a low number of loci is sufficient to produce accurate estimates.

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Distribution of *Apis florea* in different geographic locations of Pakistan

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Southeast Asia has been reported to be the primary distribution center for *Apis florea*. Oman, Iran and Pakistan with warmer climates have been reported as the habitats for

this species. However, a detailed study from Pakistan is not yet available. According to an earlier report, *A. florea* does not seem to dominate other species in the areas studied in Pakistan. It is reported to be present in plains, coastal and sub-coastal areas, tropical, subtropical, semi deserts and foot-hills throughout the year. We have reported a predominant distribution of *A. florea* in Khairpur District that has geographical coordinates 27.30N 68.50E using morphometric analysis and PCR amplification with *A. florea* specific primers. Data obtained from Oberursel data base including information on honey bee distribution from Larkana and Peshawar with altitudes 27.33N 68.18E and 34.01N 71.40E respectively. Data on the common morphometric characters for Khairpur District from this study and for Larkana and Peshawar obtained from Oberursel data base are analyzed and compared using principal component analysis and Cluster analysis. This report may extend the research on the distribution of *A. florea* in Pakistan and be useful for selection and conservation purpose.

Key words: Principal component, Apis florea, Oberursel data base, Distribution)

Effectiveness of molecular markers for the phylogeny of the Mesoamerican stingless bees genus *Scaptotrigona*

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Stingless bees are important pollinators of tropical ecosystems and some species, as *Scaptotrigona mexicana*, are commercially exploited for their honey production. Previous morphological and DNA barcoded analyses have demonstrated the existence of genetic lineages within *S. hellwegeri* and within *S. mexicana*. To further test these hypotheses we perform phylogenetic analysis of five molecular markers (mitochondrial *cox1* and *16S*, nuclear *ITS1*, *EF1* and *Argk*) with Bayesian methods of the three species of the stingless bee genus *Scaptotrigona* with Mesoamerican distribution: *S. hellwegeri*, *S. mexicana* and *S. pectoralis*. Mitochondrial rather than nuclear markers have resulted more efficient, but combining both markers we obtained higher resolution confirming preliminary hypotheses. In this sense a closer relation between *S. mexicana* and *S. hellwegeri* has been depicted as well as the existence of genetic lineages in *S. mexicana*. We advocate preventing trade and movement of those *S. mexicana* colonies resembling different species in order to conserve genetic resources of managed stingless bees for their option value for future honey market.

Keeping stingless bees in Brazil: Nation-wide survey reveals how to maximize production and income

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Beekeeping should be considered a key activity for development and conservation, as it reduces the need to exploit other natural resources, creates incentives to protect the plants visited by bees, ensures crop productivity and helps maintain plant biodiversity in natural ecosystems. In contrast to apiculture, meliponiculture (the keeping of stingless bees) has not yet developed into a large-scale global commercial business, being still considered a traditional activity with little standardization of management practices. The optimization and standardization of management practices in meliponiculture could increase productivity and income, thus making the activity more attractive to new entrepreneurs and increasing its value as a sustainable development tool. Here we profited from the great diversity of stingless bee species and local management practices found in Brazil, to identify general patterns that could aid the optimization of meliponiculture. By surveying 270 stingless bee beekeepers across Brazil, we characterized management practices and gathered data on production and sales of bee products. We then used a model selection approach to identify which management practices are better predictors of production and sales. Nearly 70% of all interviewed beekeepers had less than 50 colonies, being *Tetragonisca angustula*, *Melipona quadrifasciata*, *M. scutellaris* and *M. subnitida* the most commonly kept species. While the main bee products sold were honey and colonies, supplementary feeding and the artificial division of colonies were identified as key factors determining production and sales. Our work provides general guidelines to optimize meliponiculture and highlights the potential of this activity as a development and conservation tool.

Sneaky queen bees selectively detect and infiltrate queenless colonies

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Insect societies are characterized by advanced cooperation, but at the same time the complexity of their colonies renders them susceptible to reproductive parasitism. Recently, a genetic study on the Brazilian stingless bee *Melipona scutellaris* showed that unrelated queens frequently invade and take over colonies in which the mother queen had died. In the present study, we investigated this phenomenon using radio frequency identification (RFID) tags. We confirmed that alien queen take-overs are common within this species,

and demonstrated that mated queens actively seek out colonies without a queen to reproduce in. Furthermore, we found that queens only penetrate their target colonies in the evening, when guarding efficiency is significantly reduced. We hypothesize that this strategy reduces the chance of the queens being attacked by entrance guards, thus maximizing their chance of successful infiltration.

Genetic-morphological variability of *Melipona subnitida* (Apidae, Meliponini) in populations in northeastern Brazil suggests at least two evolutionary lineages

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The pollination by bees is essential in terrestrial ecosystems for both agricultural production and environmental conservation. The decline of these essential pollinators, as it has already been recorded in various parts of the world, will probably lead to the extinction of many other species that rely on their services. *Melipona subnitida*, a tropical stingless bee, is an endemic species of the Brazilian northeast adapted to xerophytic conditions, and exhibits great potential for honey and pollen production in addition to its role as one of the main pollinators of the Caatinga biome. To understand the genetic structure and better assist in the conservation of this species, we characterized the population variability of *M. subnitida* of six localities in the northern using geometric morphometrics of the forewing and *cytochrome c oxidase I* gene fragment sequencing. Both methodologies indicated that the variability among the sampled populations is related both to the environment in which samples were collected and the geographical distance between the sampling sites, indicating that differentiation among the populations is due the existence of evolutionary lineages. Molecular clock data suggest that this differentiation may have begun in the middle Pleistocene, approximately 396 kya. Whereas arid climates will expand their distribution, becoming of great importance for the future and the growing demand on pollinators that can be used on a large scale, the conservation of all evolutionary lineages is important since they can present differential resistance to environmental changes, as resistance to drought and diseases.

Geometric Morphometrics and Molecular Analysis of Populations of *Plebeia nigriceps* on Rio Grande do Sul, Brazil.

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Plebeia nigriceps is a stingless bee species with distribution restricted to south of South America, in regions with low temperatures. These bees are floral visitors of many native plants and are used on crops pollination in greenhouses. However, several factors may be contributing to a decrease in its diversity, including habitat loss and climate change. For a better understanding of this species population dynamics we characterized the morphological and molecular variability of *P. nigriceps* populations using two approaches: sequencing of mitochondrial DNA and geometric morphometrics of the forewings. We collected workers from 53 colonies in eight localities in South Brazil. To analyze the pattern of wing venation, we used between five to fifteen workers per colony, and 13 landmarks were plotted on each right forewing. The discriminant analysis correctly classified 96.3% of the bees on their respective population and 93.2% were correct reassigned in a cross validation test. Mantel test correlated geographic distances and the Mahalanobis distances from canonical variate analysis ($r=0.70$, $p=0.0130$), indicating that distances are in accordance to phenotypic variation. Total DNA of one worker per nest was extracted using Chelex resin. The amplification of the COI gene fragment generated products of 554 bp. Twenty haplotypes were identified and the haplotype diversity (H_d) is 0.93. These results indicate that the variability of populations is comparable to the geographical distance between the groups and proposes a certain isolation degree among them.

Evaluation of geometric morphometric techniques efficiency in two-body structures analysis (wing & head) from seven species of orchid bees

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The reliable identification of species is fundamental for any conservation studies. However, the low number of bee taxonomists, the lack of identification keys and the difficult taxonomy of the group makes bees a complicated group to work with. In the last years, some new tools and techniques have been tested in the identification of species and Geometric Morphometric (GM) has shown excellent results. Here we tested the efficiency of GM analysis of 19 landmarks plotted on right wing of seven species of Euglossine bees compared with nine landmarks plotted on the head of the same samples. Wings were placed between microscope slides and cover slip; the head was not removed from the bee body. Samples were photographed with a digital camera attached to a stereomicroscope. For the analysis, free access software were used. Our results shows that for the

wings analysis, all groups presented statistically significant differences ($p < 0.05$), and the success rate of cross-validation test was 93.33%. For the head analyzes, only the species *Euglossa truncata* x *Euglossa pleosticta* showed no statistically significant difference ($p = 0.8224$) and the success rate of cross-validation test was 76.5%. Thus, we conclude that: the GM analysis of wing was more efficient for identify and separate bee species at least for Euglossine bees and it was easier and faster to work with, in consequence of the structure be two-dimensional.

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Isolation reduces genetic diversity and connectivity but does not significantly increase diploid male production in a Neotropical orchid bee

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There is concern about the worldwide decline of bees, in which genetic factors may play a role. As populations of these haplodiploid insects suffer habitat fragmentation and subsequent isolation, theory predicts an increase in inbreeding and a concomitant diploid males production (DMP), which could lead to an extinction vortex. To test this idea, we genotyped 1245 males of *Euglossa cordata* (Apidae:Euglossini) from the Brazilian mainland and three islands using 5 highly variable microsatellite loci. Allelic richness was lowest on the most remote island 38 km from the mainland. Though the proportion of diploid males was highest on the most distant island (1.3%), differences in DMP across study areas were statistically non-significant. The degree of differentiation across localities was modest (global $F_{ST} = 0.034$, $G_{ST} = 0.153$, both $P < 0.001$). Although isolation by distance was weak, sea was revealed to be a significant barrier to inferred gene flow (partial Mantel test of distance over sea, $r = 0.831$, $P = 0.003$). Thus island isolation leads to reduced genetic variation at putatively neutral microsatellite loci, but *E. cordata* nevertheless seems to maintain allelic diversity at the sex locus, possibly because of sufficient gene flow, or because it is a locus under balancing selection, or because of the joint action of both. These and other bee species may be able to maintain sufficient variability at the sex locus to avoid DM extinction vortex, even on relatively isolated islands or habitat fragments.

Microsatellite analysis of museum specimens reveals historical differences in genetic diversity between declining and more stable *Bombus* species

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There is worldwide concern that pollinators like bumblebees undergo major declines, wherein loss of genetic diversity can play an essential role. In this project, we investigated the level of genetic diversity of seven declining and four more stable species with use of 10 microsatellite loci. Hereto we genotyped 159 museum specimens collected between 1918-1929, from 6 provinces in the Netherlands. For the stable species *B. pascuorum*, we also selected populations from two additional time periods: 1949-1955 and 1975-1990. Our results showed that the genetic diversity and population structure in *B. pascuorum* remained constant over the three time periods, but populations of declining bumblebee species had a significantly lower genetic diversity than co-occurring stable species and this before their major declines. This historical difference indicates that the repeatedly observed reduced genetic diversity in recent populations of declining bumblebee species is not caused solely by the decline itself. The historical low genetic diversity in the declined species may be due to the fact that these species were already rare, making them vulnerable for the major drivers of bumblebee decline. The results are discussed in relation to current conservation strategies, which should pay more attention to promoting gene flow and creating large, high quality, habitats for bumblebees.

Disentangling factors contributing to parasite prevalence in bumblebee communities

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Bumblebee species as well as communities might be threatened by a number of parasite species. One of those parasites, *Crithidia bombi*, a flagellated protozoan (Trypanosomatidae) lives inside the gut of *Bombus* spp. and represents a multi-host parasite. Key factors contributing to the epidemiological process are identified in order to understand, why some bumblebees species appear to be particularly prone to infections. Workers and males of three common species (*Bombus terrestris*, *B. lapidarius* and *B. pascuorum*) were sampled in 30 locations along a 300 km transect in Germany. Prevalence, type (single- vs. multiple-strain infection) and intensity of infections were determined. Workers were genotyped at eight microsatellite loci to estimate colony density and genetic diversity. Species identity, sex of bumblebees, sampling period (June, July, August) and the two-way interactions markedly affected prevalence, whereas no sex-specific differences were found for the type and intensity of infection. Colony density was positively related to prevalence,

whereas high genetic diversity was associated with low prevalence rates in *B. terrestris* only. Genetic diversity is also important at higher levels of biological organization. Highly diverse communities in terms of the number of bumblebee species do show low rates of infestation. The presence of *B. lapidarius* increases the risk of other species getting infected with *C. bombi*.

Production of Drones and Gynes from colonies of *Bombus atratus* (Hymenoptera: Apidae) bred in conditions of greenhouse and pastureland

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For over a decade the BEAS research group has been studying the biology of the native bumblebee *Bombus atratus*. Special focus has been placed on investigating the conditions that maximize the feasibility of using this species as a means for pollination of different crops, as tomato, strawberry, blackberry and paprika, among others. Captive breeding is one of the group's main achievements, but the number of resulting colonies, however, depends heavily on the availability of wild queens. This work aims at investigating the conditions that facilitate the production of new queens and drones in captivity, as a means to introduce feedback to the breeding process and reduce the capture of wild individuals. 31 colonies were evaluated in two different conditions: greenhouse and pastureland. A total of 1492 drones and 737 gynes were produced; 52% of the colonies produced drones and gynes (D&G), 35% produced drones (D) only and 13% of the colonies didn't reach the sexual phase. 16% of the D&G colonies had more than one sexual phase during a year. No colonies showed exclusive production of gynes. Greenhouse conditions produced more drones per colony (116 ± 69 in D&G colonies and 39 ± 15 in D colonies) than pastureland's (38 ± 8 in D&G-colonies and 7 ± 2 in D-colonies). However, the largest-sized drones were produced in pastureland and from colonies that produced both types of individuals (D&G-colonies). There were no difference in the amount (50 ± 13) and size of gynes produced per colony in the two types of conditions.

Proyect CIAS-1569

Effects of Stressors on the Behaviour of Bee Pollinators

Organizers: Stephan Wolf, Beth Nicholls

Given the immense ecological and economic importance of pollinators, recent bee declines are cause for great concern. Pathogens, pesticides, mal-nutrition and their potential interactions constitute major challenges to bee populations. In most cases the effects of such stressors are likely to be subtle, affecting colony performance in cryptic ways, thus making such effects difficult to study.

Bee colonies are highly complex units, the functioning of which is dependent on the synchronised actions of hundreds, sometimes up to thousands of individuals. Colony success therefore depends on the behavioural performance of its members. Sub-lethal, and hence easily overlooked, effects on the behaviour of individual bees may multiply, resulting in mal-performing colonies and declining populations. Consequently emerging research concerning the sub-lethal effects of external stressors represents an important and challenging area of pollination science. This symposium will focus on the behavioural effects of external stressors on individual bees, and how these may be manifested at both a colony and population level, as well as considering the dynamics of the stressor itself.

Invited speakers: Geraldine Wright, Lèa Tison, Nadege Forfert & Robin F. A. Moritz, David Baracchi

Interaction of dietary protein and neonicotinoids on the survival and nutrient balancing of *Bombus terrestris*

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Poor nutrition, diseases and exposure to pesticides in modern agriculture are all like to contribute to the decline of wild pollinators. We know surprisingly little about the nutrition of wild pollinators and how they obtain sufficient nutrition in modern agricultural landscapes. Several controversial studies have shown that colonies of buff-tailed bumblebees, *Bombus terrestris*, exposed to neonicotinoids in food have impaired brood rearing, compromised foraging, and poor worker survival. Nutrition could be an important mitigator of the survival of wild pollinators when they are exposed to low doses of pesticides. Here, we used the Geometric Framework for nutrition to identify the optimal nutrition of adult foraging workers of *Bombus terrestris* for diets composed of protein and carbohydrates. We also we tested nutrition the bumblebee's susceptibility to the neonicotinoid pesticide, imidacloprid. Using a paired-diet design on individual workers, our experiments identified the intake target (IT), or optimal ratio of protein-to-carbohydrate (P:C) to be 1:170. When bees were exposed to imidacloprid, they ate significantly less food. As expected if pesticides increased the demand for nutrients, the bumblebees' IT shifted towards a diet higher in protein. Contrary to our expectations, however, bees fed diets containing any protein had a significantly greater risk of dying than bees fed sucrose alone. We predict that bees that consume neonicotinoids face a trade-off between consumption of protein that they need for somatic maintenance and egg laying and survival. Our data identify a possible mechanism for the previously observed slow declines of wild bee populations exposed to neonicotinoids.

Sublethal doses of neonicotinoids affect navigation, foraging behavior and social

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Honeybee foragers may be exposed to neonicotinoids during their foraging flights via pollen, nectar and guttation drops. It has already been observed that neonicotinoids compromise navigation in honeybees. Yet, the colony relies on the foragers' ability to locate food sources and bring pollen and nectar back to the hive. Using harmonic radar tracking we show that the homing flight component in treated bees cannot be perform as precisely as in the control bees (Fischer et al. Plos one March 2014). The waggle dance may also be affected by sublethal doses of neonicotinoids since they interfere with the nicotinic synaptic transmission in the central nervous system, possibly altering social communication processes. We trained foragers from two colonies of honeybees *Apis mellifera carnica* in observation hives to two separate feeders. One group (from the experimental colony)

foraged 4 weeks on a sucrose solution containing a sublethal dose (0.02 mM) of thiacloprid, and another group (from the control colony) foraged over the same time at a feeder containing only sucrose solution. Navigation abilities of these bees were tested in the same kind of catch-and-release experiment as in the Fischer et al. study. In addition, bees' waggle dances were videorecorded and the electric fields of the dancers were measured. The traffic at each feeder was estimated using light sensitive detectors at the feeders' entrances. We found that chronic exposure of a colony to a field-relevant dose of thiacloprid in field-realistic conditions affects honeybees' social communication and foraging behavior.

The Neonicotinoid Pesticide (Thiamethoxam) affects foraging behavior of honey bees

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This study was performed to determine the effects of sub-lethal dosages of Thiamethoxam on Anatolian honey bee (*Apis mellifera anatoliaca*). The visitation rate and foraging abilities of free flying bees on artificial flower patches were investigated. Three experiments were performed with 12.0 ng thiamethoxam (40% of the LD50), 3.0 ng thiamethoxam (10% of the LD50) and 0.6 ng thiamethoxam (2% of the LD50). The flower color sequence visited by each bee and also number of trips made from hive by a bee was recorded. Thiamethoxam dosage affected the number of bees that returned to a foraging table after being fed pesticide. At a dose of 40% LD50 only 4% of the bees returned to the flower patch after their release. Furthermore, a dose as small as 10% LD50 was sufficient to shut down foraging with only 22% of the bees returned to forage. Low doses of 2% of LD50 of Thiamethoxam had no discernable effect on flight, but reduced the effectiveness of foraging bees, more bees deviated from optimal foraging than in controls when complex learning was required. The results here showed that the return rate of foraging bees was significantly lower for pesticide-fed bees at doses as small as 1/10 LD50 and foraging decision to distinguish between high and low sucrose nectar was impaired at doses higher than 1/50 LD50. In conclusion the sub-lethal doses of Thiamethoxam affect foraging behaviour of honeybees.

Do neonicotinoids affect social interactions of a contaminated bee within a small group of nestmates?

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Since the last decades, neonicotinoids have become a frequently used class of agricultural insecticides. Their toxicity to bees has been claimed to be a major stressor inter-

fering with the declined of honeybees. Field realistic doses have been shown to cause a wide range of adverse sublethal effects in honeybees including impairment of orientation, homing failure and nervous system damage. To go beyond testing the impact of pesticides on individual workers, we performed a quantitative approach of social interactions (i.e. antennating and trophallactic events) of individuals that had been exposed to an acute sublethal dose of thiacloprid, a neonicotinoid in a small group of nestmate workers under laboratory conditions. Bees fed with the agrochemical shown less social interaction with their nestmates. This result was complemented with a social network approach in which the centrality of the contaminated bees was quantified which resulted in the reduction within the network. Hence we believe that non-lethal doses of those pesticides can lead to the disruption of the communication system of the colony which may interfere with colony functioning.

The impacts of pesticides on bumblebees: from individual behaviour to colony function

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Bees are essential pollinators of many crops and wild plants. Whilst pesticides are suggested to be one factor that could be driving bee declines, a key question is to what extent exposure to field-realistic levels of pesticides might have significant (sublethal) impacts on individual behaviour, colony function and reproductive success. Here we report results from a series of laboratory and field studies of pesticide exposure on bumblebee (*Bombus terrestris*) behaviour, conducted as part of the UK Insect Pollinators Initiative. Chronic field-level exposure to two pesticides, a neonicotinoid (imidacloprid) and a pyrethroid (lambda-cyhalothrin), impairs natural foraging behaviour and increases worker mortality leading to significant reductions in brood development and colony success. Neonicotinoid exposure has both acute and chronic effects on overall foraging activity, including changes to forager preferences for the flower types they visit to collect pollen and significant reductions in individual pollen collection efficiency, and may also influence navigation. These individual impacts have clear knock-on effects for forager recruitment, worker losses and overall colony productivity. Moreover, we provide evidence that combinatorial exposure to pesticides increases the propensity of colonies to fail. Combining empirical data with modelling highlights how sublethal stress on individual bees can disrupt colony function and lead to colony failure. Firstly, a stressor must have a chronic impact (over a period of several weeks) before we see any noticeable effect. Secondly, we show how a stressor that impairs colony function can cause Allee effects making colonies especially susceptible to failure at earlier points in their lifecycles.

Evaluation of pesticide toxicity to the honeybee: a new approach of the hazard quotient

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Chemicals are used in agriculture (fertilizers, pesticides) and can be potentially found in the environment. In order to know their impacts on the non-target organisms and to limit their undesirable effects, these products are evaluated according to several tests. One of the first steps is the establishment of the hazard quotient to evaluate the level of risk presented by the use of a molecule to an organism. This ratio is obtained by comparing the toxicity of the molecule to this organism and the quantity or the concentration to which it can be exposed in the environment. In honeybee tests, the environmental exposure is based on the amounts of active substance spread on one hectare of cultivated surface. This factor does not take into account the amounts that can be in contact with a forager during pulverization and thus does not enable a real evaluation of the risk. Our study proposes a new approach to evaluate the exposure and the potential toxicity of the pesticides to honeybee. We tested 20 of the most common commercial pesticide preparations on bees in order to know the rates of residues deposited on the bees during the pulverization in the field. This enabled us to determine an exposure surface of around 1 cm² per honeybee and then to convert an amount per hectare into an amount per bee. This new approach of the risk is more accurate and could become a tool for decision-making aid in the evaluation of the toxicity of pesticides.

Honey bee development and physiological performance as influenced by field exposure to radio frequency electromagnetic radiation

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Among the suspected causes in the lost honey bee colonies mantra, radiofrequency electromagnetic fields (RF EMF) often are mentioned. Bees can detect electromagnetic fields and respond to these fields. Effects of RF EMF on bees can thus not be ruled out, and many people are concerned about possible effects on bees as well as other organisms including man.

An experiment was conducted in 2011 in which twenty honeybee colonies housed in fully metal free styropor six frame hives were subjected to the RF EMF of a nearby antenna, shielded (control) by placing in a Faraday cage, or exposed by placing in a com-

parable cage with plastic instead of metal netting. Field strengths inside and around the cages were registered and analysed.

Our research shows that individual bees from the exposed and the control group did not significantly differ in (1) developmental success from egg to larva to adult bee, (2) morphometric and physiological parameters at emergence, (3) flight performance in their adult stage, (4) longevity of worker bees in the colony. At colony level, we succeeded to keep the exposed and non-exposed colonies comparable: they had comparable development (production of brood and young bees) and a comparable number of queen loss incidences. The experimental set-up and scale did not allow us to statistically evaluate winter survival of colonies; a seeming difference in winter survival urges for additional research. Our results suggest that RF EMF is a non-probable negative factor for individual honey bees and for honey bee colonies.

Results from a National-wide monitoring project in Italy and the Bee Emergency Service Team

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The BeeNet monitoring network is active since 2011 with 303 apiaries (10 hives each) located in all the Italian regions. Total mortality was not correlated to any of the investigated parameters. However, the total mortality almost doubled in apiaries infected by *Nosema* compared to the negative ones. Furthermore, *Varroa* infection is directly correlated to ABPV, while Winter mortality is negatively correlated to the percentage of beebread raw protein. Overall 50.4% of beebread samples analysed was positive to at least one active ingredient. In 2012, the infection by *N. ceranae* was between low and medium; only in 3.4% of the samples, more than 10 million *N. ceranae* spores per bee were detected. *N. ceranae* was present in all Italian regions, neither *N. apis* nor *N. apis/N. ceranae* co-infection were detected. DWV, ABPV and CBPV were detected in Italian apiaries in different combinations. DWV was present in almost all samples (96.7%) and in 40% of cases exceeded 10 million viral copies per bee. Bee Emergency Service Team (BEST) has been established for field intervention, samples and data collection, and epidemiological investigation. Data so far obtained (72 reports in 2013) indicate that pesticides were the primary cause of mortality in 15 reports, disease (especially viruses) in 2, while interaction between pathogens and pesticides has resulted in the loss of bees in 3 cases. Until now the pesticide residues were found in 44 samples, of which 63.6% tested positive. The active ingredients found in different matrices were 22, including 13 insecticides, 8 fungicides and 1 herbicide.

BEEHAVE: modelling multifactorial causes of honeybee colony losses

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Honeybee colonies can be subject to multiple stressors like parasites (e.g. varroa mites and varroa transmitted viruses), insufficient forage availability and pesticide exposure. We developed a honeybee model that integrates colony dynamics, agent-based foraging in realistic landscapes and population dynamics of varroa mites, acting as vectors for viruses (Deformed Wing Virus and Acute Paralysis Virus). We explore the potential impact of pesticide applications by increased mortality rates of foragers and in-hive stages and how this is affected by food availability in the landscape. Our results suggest that the timing of the pesticide exposure is crucial and that the colony is more vulnerable to forager losses if it is already under food stress.

The BEEHAVE model is available for free download at <http://beehave-model.net/> and comes together with a detailed model description and a user manual.

Infected bumblebees self-medicate by increasing the ingestion of nectar toxins

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In the wild, *Bombus spp.* may contract infections of the trypanosome parasite *Crithidia bombi* from their nestmates or contaminated flowers while foraging. *C. bombi* has a wide range of impacts on worker bumblebees, inducing a costly immune response, increasing mortality under food stress, and reducing learning and foraging efficiency. As consequence, any mechanisms that reduce infection intensity in a forager would therefore improve the health of its colony. Evidence that secondary metabolites in nectar have antimicrobial activity suggests that bumblebees may derive a benefit from the consumption of nectars rich in these metabolites. While some insects are known to shift their diet to toxin-rich food to counteract infections, there are no reports of active self-medication (pharmacophagy) in pollinators. We tested whether nicotine, a nectar secondary metabolite common in some bee-pollinated plants, could reduce pathogen load in bumblebees infected by *C. bombi*. We first studied whether the consumption of nicotine by bumblebees reduced their parasite load, and then, whether nicotine directly reduced the infectivity of the protozoans. Finally, we allowed healthy and infected bees to choose their diet from plastic flowers in an artificial arena to test for a nicotine preference among infected individuals. Our results demonstrated that artificial nectar containing a natural nectar alkaloid reduced the severity of a gut infection in bumblebees. More interestingly, our behavioural test showed that infected bees actively self-medicate themselves by shifting their diet towards non-nutritive secondary metabolites. This finding raises the possibility that pollinators play an

important role in maintaining secondary metabolites in plants.

Effects of parasitism and immune challenge on flight activity and survival in honey bees tracked with optical counters

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During their life, honey bees can be exposed to numerous parasites. Different studies have identified the consequences of parasitism on bee physiology and survival. However, parasitism and the associated immune response can also induced changes in the host behaviour, which are either beneficial to the parasite or the host or simply a non-adaptive byproduct of parasitism. Notably, one major finding has been that parasites and immune challenge induce precocious foraging, but less is known about the actual foraging activity of parasitized bees and whether mounting an immune response would also affect the flight activity. Therefore, we exposed honey bee workers to the parasite *Nosema ceranae* or to an immune challenge, and tracked their flight activity over their lifetime with optical counters. We found that bees responded differently to each stress: both *Nosema*-parasitized and immune-challenged bees performed a lower number of daily flights compared to control bees, but the duration of their flights increases and decreases over time, respectively. Overall, parasitized bees spent more time in the field each day than control bees, and inversely for immune-challenged bees. Finally, *Nosema*-infected bees had a lower survival, but immune-challenged bees had a survival similar to control bees likely due to their restricted activity. We will discuss how those different behavioural modifications could be adaptive phenotypes. This study provides new insights into the evolution of host/parasite interactions in bees and how parasites and immune stress can affect colony dynamics.

There and back again? – The effects of emergent pathogens on honeybee orientation flights

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Detailed and reliable knowledge about one's environment is an essential pre-requisite for successful foraging of central-place foragers such like honeybees. The acquisition of environmental information is vital for foraging honeybees affecting its success as a forager. Orientation flights of novice foragers have been shown to follow a scale free (fractal) spatial distribution (Levy-flights) optimizing the exploration of a novel environment.

In various species the loss of fractal complexity in behavioural sequences leading to

more stereotypical i.e. less optimal behaviour, was shown to be negatively associated with various forms of stress including health status. However, the effects of stress, such as pathogen-infection, on the spatial movement patterns of animals remain unknown. Here we study the potential effects on honeybee orientation flights of two fundamentally different types of honeybee pathogen: *Nosema ceranae*, an obligatory gut-parasite acquired by adult bees only, and various Varroa-associated viruses including Deformed Wing Virus (DWV) and Varroa-Destructor Virus (VDV), transmitted during the early development of a bee.

Sub-lethal effects on the way bees equip themselves with the vital knowledge about their environment may have marked consequences for both individual bees and the colony as a whole, yet the causes of which are highly cryptic. We used harmonic radar tracking to explore the effects of emergent bee-diseases on their hosts' orientation flights. In addition to implication for honeybee conservation, investigating the spatio-temporal fractal patterns of individual movement decisions in context of health status may provide novel insights into effects of stressors on animal behaviour in general.

The influence of mass-flowering oilseed rape and semi-natural habitats on honey bee foraging behaviour

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The increasing demands for insect pollinated crops and high recent losses of *Apis mellifera* colonies raise concerns about food security. Agricultural ecosystems can provide mass food resources for honey bees but also pose a high exposure risk to chemical pesticides or transgenic traits. Information about spatial and temporal intake of pollen and nectar from mass-flowering crops helps to understand how landscape structure modulates foraging behaviour of honey bees. We have used the spatial information provided by bee dances to analyse how the amount of oilseed rape and semi-natural habitat area affects foraging distances and whether there are preferences for these habitats. It turned out that pollen foraging distances decreased with increasing oilseed rape area in the landscape in spring. Increasing cover of semi-natural habitat areas close to the hive reduced pollen foraging distances in spring and summer. Pollen foraging visitation rates per area were significantly lower on crop than on semi-natural habitat, grassland, oilseed rape and gardens. Our results suggest that colonies aim for a diverse pollen diet throughout the year with semi-natural habitat being one of the most important pollen sources. Mass flowering rape and semi-natural habitat both have the potential to reduce overall foraging distances and consequently energy efforts of honey bee colonies. Further findings allow estimating the distance-related exposure risk of honey bee colonies to pollen and nectar from rape fields treated with systemic or other insecticides.

Foraging and pollination behaviour of *Chalicodoma rufipes* L. on *Cajanus cajan* L. Mill sp. (Fabaceae) flowers at Dang (Ngaoundéré, Cameroon)

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To evaluate *Chalicodoma rufipes* impact on fruit and seed yields of *Cajanus cajan* L., its foraging and pollinating activities were studied in Ngaoundéré for two seasons. Observations were made on 340 flowers each year and divided in three treatments. The treatments included unlimited flowers access by all visitors; bagged flowers to deny all visits and limited visits by *C. rufipes* only. *C. rufipes* worker seasonal rhythm of activity, their foraging behaviour, their pollination efficiency, the fruiting rate, the number of seeds per pod and the percentage of normal seeds were evaluated. Results show that *C. rufipes* foraged *C. cajan* flowers throughout the whole blooming period. That bee intensely harvested pollen and nectar. The mean foraging speed was 11.50 flowers per minute in 2010 and 12.45 flowers per minute in 2011. The fruiting rate, the number of seeds per fruit and the percentage of normal seeds of unprotected flowers were significantly higher than those of flowers protected from insects. Through their pollination efficiency, that bee provoked a significant increment of the fruiting rate by 95.38% in 2010 and 96.72% in 2011, as well as the mean number of seeds per fruit by 04.28 in 2010 and 04.93 in 2011, and the percentage of normal seeds by 50.72% in 2010 and 61.79% in 2011. The installation of *C. rufipes* nests close to *C. cajan* fields could be recommended to increase fruit and seed yields and to alleviate poverty in that region.

Effects of biocontrol agent *Gliocladium catenulatum* on the behaviour of honeybee, *Apis mellifera*

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BICOPOLL is a European study on protecting organic strawberries from the most important disease, the grey mould (*Botrytis cinerea*), using biological control. Honeybees are used to deliver biological fungicide Prestop® Mix (PM) containing biological control agent fungi *Gliocladium catenulatum* to the flowers to provide control of diseases. Bees gathered PM in a dispenser exit placed at the hive entrance and delivered it to the flowers. The effect of PM on the behaviour of the honeybee was studied. The behaviour of foragers in dispenser was observed by two video cameras. PM reduced the number of direct crossings of the dispenser and bees were trying to avoid it. Changes were the most obvious in the first hour, and then subsided. In homing ability experiment the treated and untreated bees were released from a distance of 40 m from the hive. Results showed prolonged flights in bees that were exposed to PM but did not affect returning rate until evening. The effect of exposure to PM on learning and memory was tested using olfactory condition-

ing of proboscis extension reflex (PER). Bees were conditioned to the olfactory stimulus once and tested for PER response by presenting odour alone 1 or 12 min after conditioning. Before learning experiment responsiveness to ascending sucrose solution was tested to see whether bees differ in sucrose responsiveness which affects learning abilities. The results did not confirm any effect. We suggest some improvements of the dispenser to decrease affects of PM on behavior of honeybees.

Global evaluation of pollinators in agricultural crops

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Crop pollination by insects is increasingly recognised as a central ecosystem service provided to a large number of crops worldwide and contributing to the increase and stability of production. While honeybees have been the main pollinators integrated in management practices, other pollinators such as wild bees have been shown to substantially contribute to the production of major crops such as almond, cherry, strawberries and sunflower, among others.

Pollination studies have been carried out for most crop species, sometimes back in the 50s, but most of this literature, particularly before the 90s, is difficult to access. We have compiled a database including as many papers as we could find on the pollination of more than 100 crops. Information collected included pollinator and visitor communities, what they forage for, and their impact on fruit and seed production. Building on this past research, we aim to give an overview of the importance of pollinator communities in crop production. We will specifically focus on the following questions: What is the diversity of pollinator populations across crops and regions of the globe? How much do crops depend on pollinators for their production?

Physiology

Organizers: Anton Stabentheiner, Helmut Kovac

Bees live in a world of complex interactions with their environment. In order to understand these interactions the knowledge of the underlying physiological processes, from the whole animal down to the cellular and sub-cellular level, is indispensable. When bees forage energetic investigations have to be combined with behavioral considerations to assess questions of individual and colony level energetic optimisation. During the social life inside a colony nutrition and immune defence, hormonal and genetic control of development, and the hormonal and genetic control of physiological processes of the brood and the adults come into focus. At all levels of physiological investigation the flow of information between different physiological and behavioral disciplines promotes the gain of knowledge. The physiology symposium aims at bringing together bee physiologists of all disciplines and taxa, from environmental and behavioral physiology, energetics, respiratory physiology, nutrition, mechanisms of immune defence, and endocrine regulation.

Invited speakers: Reet Karise, Christian W. W. Pirk

Nutritional aspects of honeybees, *Apis mellifera*- Macronutrient, secondary metabolite and stressor interactions

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Honeybees need nectar and pollen for individual and colony growth and development. Proteins and carbohydrates are the most abundant macronutrients in their diet. To understand what constitutes a healthy diet for honeybees, it is essential to know the interactions between dietary components like proteins and carbohydrates, secondary plant metabolites like nicotine and ECGC (green tea), and stress factors like pesticides or environmental conditions. We show that bees are able to adjust their intake of macronutrients when they encounter a nutritionally variable landscape. Under brood-free conditions the intake target reflects the needs of the workers. When offered more readily available proteins, either as protein hydrolysate or amino acid mix they eat less protein. Furthermore, increased protein intake leads to increased survival in bees exposed to nicotine at cold temperatures. However, as proteins might result in oxidative stress we tested if a supplementary antioxidant might counteract this. ECGC, the active component in green tea, showed only a slight positive effect at low dosage, but a negative effect at high dosage, suggesting that excess consumption of ECGC might carry negative effects. The interactions between the different dietary components, secondary metabolites, and stressors make this a complex and dosage dependent system. Only by understanding what constitutes a healthy honeybee diet will one be able to conserve honeybees and ensure their continued contribution to ecosystem services and food security.

How to stand the heat: Adaptations of stingless bees to extreme environmental conditions in the Brazilian tropical dry-forest

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The tropical dry-forest in northeastern Brazil, the Caatinga, is characterized by elevated ambient temperatures that, sometimes, far exceed the lethal threshold for most insects. These thermal conditions and the sporadic, short, and in some years virtually inexistent rainy season render the Caatinga a hostile environment for eusocial bees with perennial colonies. Due to the lack of floral resources and water for potential evaporative cooling over long periods of the year, only few eusocial bee species naturally occur in this ecoregion. Even the (introduced, Africanized) honey bee, which is one of the most adaptable and, thus, successful bee species, abandons parts of the Caatinga in years of extreme drought. To understand, how the few existent eusocial bee species are able to survive and

successfully reproduce in this environment, we studied adaptations at various levels of the stingless bee *Melipona subnitida* (Apidae, Meliponini). Our results show (1) the fundamental importance of the thermal isolation of the nest cavity-surrounding material for reducing temperature oscillations within the nest, which points to an adaptive selectivity for certain nesting sites. (2) In the hottest months, the colony actively cools down the storage pot-area, which results in a temperature reduction in the entire nest, and maintains the brood-area below ambient temperature. (3) The colony down-regulates brood-production in periods without rainfall and, hence, with few or no floral resources. In consequence of this reproductive diapause, colonies economise on the stored food (nectar and pollen) and are able to survive the long periods of dearth in the Caatinga.

The upper lethal temperature of two European honeybee races, *Apis mellifera carnica* and *A. m. ligustica*

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The two subspecies *Apis mellifera carnica* and *Apis mellifera ligustica* are closely related and live in neighboring climatic regions. The Carnolian bee is the honeybee of the temperate Central Europe and the Ligustica bee is native to the Mediterranean South Europe. We investigated whether these two subspecies developed adaptations to their local climate conditions. For the experiments about 20 forager bees of each race were caught after leaving the hive and put separately in a divided cage and transferred into an incubator. During experiments the bees were provided with 1 M sucrose solution. Starting with an initial temperature of 30 °C, the temperature was increased at a rate of 0.4 °C per minute to a maximal temperature between 46 °C and 56 °C. When the target temperature was attained, they remained for five minutes and afterwards the temperature was quickly reduced to 30 °C and the bees stayed for further eight hours in the incubator. The number of living and dead bees was counted in two hours intervals. The mortality was plotted in dependence on ambient temperature and from the obtained sigmoidal curves the temperature was determined, where 50% of the bees were still alive (lethal temperature = LT50). The LT50 after eight hours was 1.4 °C higher in the Ligustica bees and significantly different between the two subspecies (Carnica: LT50 = 50.3 °C; Ligustica: LT50 = 51.7 °C; $p < 0.02$; $N = 21$ trials; ANOVA). Results revealed that the two closely related subspecies differ in their thermal traits.

Linking energetics and thermoregulation of the foraging honeybee: from experiment to a heat exchange model

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Heterothermic insects like honeybees have to cope with an enormous heat loss during foraging because they have to keep their body temperature high to stay ready for immediate flight and to promote fast exploitation of resources. During fine weather they prefer foraging in sunshine to gain additional heat from solar radiation. Solar heat may be invested to speed up foraging or to save costs. The type of flower or reward influences thermoregulation. However, the balancing of body temperature regulation with the own energetic effort and heat gain from the environment is not well known. Simultaneous measurement of body surface temperature, energy turnover and environmental parameters in bees foraging from artificial flowers uncovered a relationship considerably more complex than expected. Therefore, standard models of heat exchange did not provide satisfying results. We developed a model which accounts for the fact that in honeybees several parameters of the heat exchange process like the exact surface area for heat exchange, respiratory convection, and effective absorptivity or emissivity of solar and infrared radiation cannot be determined well enough experimentally. The model allows estimation of energy turnover from body temperature and environmental parameters to ~6-7 mW (1 SD of residuals). The model covers a variation (in measured data) of metabolic heat production from ~4-85 mW, of T_a from 14.8-37.5 °C, and of global radiation from ~4-920 W m⁻². Our comprehensive dataset allowed the direct verification of the accuracy of this advanced model for each stay at the artificial flower. Supported by Austrian Science Fund FWF-P20802.

The flow of nutrients in a honey bee colony: The importance of unequal shares

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The special feature in the super organism honey bee is that foragers are collecting the nutrients, but other worker bees are engaged in processing and storing these nutrients. The most important mechanism of transferring different liquids within the colony is trophallaxis. Larvae of different ages and sex receive distinct composed diets and different amount of food. We wanted to know if the differentiation in feeding is continued among worker bees after emerging as adults. As a first step we investigate this complex mechanism in an artificial environment on individual bees. We designed different assays to investigate food sharing in isolated arena experiments. This experimental set-up allows us to study the direct interaction between given partners of defined age and developmental status. For arena experiments we found nurse bees to be perfect as donor bees. We also could show that although we only fed sugar water prior and during the experiment; transferred protein was always measured in the recipient bees. Bees of different ages received

different amounts of food; in a set-up with more than one recipient we could show that forager bees were provided best via trophallaxis compared to younger bees. This unequal food share might have an important effect in the distribution of natural and artificial food sources as well as on the spread of pathogens and the pesticide load of individual bees. Different loads of e.g. pesticides can affect the different members of a honey bee colony to a varying extend and might cause different patterns of danger.

Major royal jelly proteins in honeybees

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In the honeybee *Apis mellifera*, female larvae destined to become a queen are fed with royal jelly, a secretion of the hypopharyngeal glands of young nurse bees that rear the brood. The protein moiety of royal jelly comprises mostly major royal jelly proteins of which the coding genes (*mrjp1-9*) have been identified on chromosome 11 in the honeybee's genome. We determined the expression of *mrjp1-9* among the honeybee worker caste and the sexuals in various body parts. Specific *mrjp* expression was not only found in brood rearing nurse bees, but also in foragers and the sexuals. Whereas the focus of the expression of *mrjp1* to 7 lies clearly in the heads of brood rearing nurse bees AND foragers, the expression of *mrjp9* was high in the heads, thoraces and abdomen of almost all female bees, suggesting a function irrespective of body section. This completely different expression profile suggests *mrjp9* to code for the most ancestral major royal jelly protein of the honeybee. In addition to the expression studies we determined the level of conservation of the *mrjp* genes including both the species and subspecies level. We found conserved signalling domains in almost all *mrjps* supporting the expression data that MRJPs fulfil a crucial role in the honeybee.

Amino acid and carbohydrate tradeoffs by honey bee nectar foragers.

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Honey bees are important pollinators, requiring pollen and nectar for nutrition. Nectar is rich in sugars, but contains additional nutrients. Here we focus on how nectar foragers evaluate amino acids (AAs). We tested the preferences of free-flying foragers between 20 AAs. Bees were offered purified AAs at 0.1 % w/w in sucrose solutions in an artificial meadow. We found consistent preferences amongst the 20 offered AA's, and essential AAs

were preferred over nonessential AAs. The preference correlated negatively with a deviation in pH values, caused by the dissolved AAs. Next, we quantified tradeoffs between attractive and repellent AAs at the expense of carbohydrates in nectar. Bees were attracted by Phenylalanine, willing to give up 94 units sucrose for 1 unit AA. They were repelled by Glycine, with 114 units sucrose required to offset the repellent effect of 1 unit AA. In addition, we tested the effect of AA nutrition on forager homing performance. Low sucrose nutrition reduced flight performance of bees, with important methodological implications for homing studies that evaluate the effect of substances that may affect imbibition of sugar solution. However, no indication was found that AAs affected foraging behavior directly, nor indirectly by means of affecting the sucrose uptake of foragers. Furthermore, in a no-choice context, caged bees were indifferent to the AAs in sucrose solution. The low AA concentrations in nectar relative to pollen suggest a limited role in bee nutrition. However, bee AA preferences could mediate plant-pollinator interactions, with plants taking advantage of pollinator biases.

Optimizing drone fertility with nutritional supplements to honey bee colonies during spring

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Spring honeybee colony feeding is essential for colony build up in northern apicultural regions. Knowledge on the impact of this feeding on drone production and quality of their sperm production is not well documented and could result in poor fecundation of early-bred queens. In the present study we measure the impact of sucrose syrup and/or protein supplement feeding to colonies in early spring. Drones were reared under different colony nutritional conditions and mature drones were evaluated for size, weight, semen quantity and quality (sperm count, motility and viability). Our results show significant increase of drone weight and abdomen size when colonies are fed sucrose and a protein supplement. Colonies without any additional nourishment had significantly less semen volume per drone and lower sperm viability. Our work shows that spring honeybee colony feeding with sucrose syrup and protein supplement is important to enhance drone quality and eventually insure adequate queen bee mating.

Kaolin as inert material in bio-pesticide formulations supplements the hazard to useful insects

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Kaolin is an inert material with a broad range of applications, e.g. non-toxic insecticide, fungicide and additive substance to many plant protection products in agriculture. Inert materials and biocontrol agents as alternatives for classical chemical pesticides are gaining more and more attention especially in the context of the European IPM directive 2009/128. In this project we investigated the safety of two model inert materials, namely kaolin, that is used commercially against different pest insects and wheat flour that we considered as safe, and of two biocontrol agents, namely the biofungicide Prestop Mix and the bioinsecticide BotaniGard. We tested for lethal and sublethal effects on workers of the bumble bee *Bombus terrestris* L.

Kaolin and Prestop Mix treatments increased cuticular water loss. Kaolin affected also survival of bumble bee foragers. Prestop Mix (containing kaolin) had no effect on survival, we suppose because of lower concentration of kaolin. BotaniGard also decreased the survival as it should do because of insecticidal fungus *Beauveria bassiana*.

Our data indicates that products as inert materials and biocontrol agents which are used in IPM and organic farming systems also need adequate risk assessment testing as classical pesticides. Specifically, the data demonstrated negative effects of kaolin and BotaniGard to entomovectoring bumble bees.

Dose-dependent effects of stacked Bt maize pollen on *in vitro* reared honey bee larvae

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In agricultural landscapes honey bees (*Apis mellifera*) are exposed to a number of different genetically modified (GM) pollen. To assess environmental risks of transgenic crops standardised first tier risk assessment studies are highly needed. Here we conducted an *in vitro* larvae rearing experiment to evaluate the potential effects of GM Bt maize pollen on honey bee larvae. The pollen was added to an artificial diet to mimic the natural exposure pathway. It was fed in increasing quantities to test for dose-dependent effects. In addition to the survival we also measured several sublethal effects; digestibility, prepupal weight, and developmental delay. Bt pollen was digested equally well as two control maize pollen types. Increasing amounts of Bt pollen did not have a negative effect on the survival of honey bee larvae, but prepupal weight decreased with higher doses of Bt pollen when compared to the multifloral pollen treatment. We detected no negative effects on development of bees that were fed Bt pollen. This study highlights the importance of including

multiple assessment endpoints, as responses may differ on a sublethal level. The presented *in vitro* approach follows the natural exposure pathway to insecticidal proteins, i.e. via the pollen, and as such it can also be used to test the effects of pollen containing systemic insecticides. We propose that in Environmental Risk Assessments (ERA) of transgenic plant products a range of field realistic doses is tested, similar as is done in toxicological studies of chemical plant protection products.

How hemolymph protein vitellogenin shapes honeybee health

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A protein called vitellogenin is associated with multiple positive health effects in the honeybee. These include increased resistance against oxidative stress and a slowdown of immunosenescence. A dramatic increase in vitellogenin is a hallmark of changes that prepare the honeybee for wintertime or drought. What are vitellogenin's molecular mechanisms in supporting longevity in the honeybee? We show vitellogenin can shield insect cells against oxidative damage via binding directly to cell membrane, and the protein recognizes damaged cells with increased phosphatidyl serine lipid on their surface in a putatively anti-inflammatory manner. Not only does vitellogenin recognize patterns of damage in honeybee's own cells, but it also binds to pathogen cells. Thus, we introduce honeybee Vg as a damage and pathogen pattern recognition receptor. Because insects lack antibody-producing immune cells, such a recognition receptor protein can potentially be highly valuable as a defense tool. Our results can shed light on the rapid evolution of vitellogenin in the honeybee and other insects, since this evolution might reflect the need to adapt to recognize new or evolving pathogens.

Queen reproductive quality based on colonies development and vitellogenin profiles, in Africanized honeybee, *Apis mellifera*, with different weight at emergence.

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The influence of honey bee queen weight at emergence in her reproductive capacity is long time questioned. Here we examined the importance of this marker for predicting colonies performance and queens physiological characteristics, based on genetics analysis. Initially, we verified the influence of queen's weight on brood viability, development and productivity of the colonies. It was established two colony groups: one with six light queens (<180mg) and another with six heavy queens (> 200mg). The colonies with se-

lected queens were monitored biweekly to estimate the eggs, pollen supply and brood viability and quantity. These evaluations revealed that colonies with heavy queens showed significant higher average of brood viability and area, beside the pollen rate. Afterwards, the queen reproductive quality was checked by physiological analysis. For that, a new set of queens was produced, grafting worker larvae at the same age. The hemolymph and the abdominal fat body from each sample were collected from each group (light and heavy). Subsequently, hemolymph protein profiles and Vg gene expression were analyzed. The RT-PCR showed that heavy queens had significantly higher mRNA levels of *vg* than light queens, at the abdominal fat body. However, the SDS-PAGE indicated that the Vg availability in hemolymph is higher in light queens. Despite further analysis are still necessary for a better understanding of the queen physiology, our data is an encouraging indicative of the superior physiological quality of heavy queens and the promising selection index in breeding programs.

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Do honeybees choose diet under parasitic infection?

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The multiple threats that affect the honeybee health are studied since ages, but quite little attention is given to the possible changing in their behavior and especially in their diet. There are many plants that help the honeybee to strengthen their fight with the disease, to boost their immune system, but not always this could be a solution. Therefore, they have to defend themselves as much as it's possible with their own 'weapons' such as immunity or collection of antimicrobial substances – intake of propolis.

The term of 'defend' may be a little rough, but it is very well suitable to honeybee behavior. We here try to explain what the honeybee does in case of a disease, if the honeybee changes the diet according to *Nosema ceranae* infection. The *Nosemosis* infection occurs very easily inside the colony for the simple reason that the honeybee is a social insect that lives in colonies. Therefore, it is expected that self-medication to be a good method of treatment, such as choosing certain type of honey, according to their health status. As our experiments proved, the sunflower honey is preferred by the honeybees among other types of honey, as the degree of infection is getting higher.

Effects of *Tropilaelaps mercedesae* mite on immune response of *Apis mellifera*

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Tropilaelaps mercedesae is the most serious parasite of European honey bees (*Apis mellifera*) in Asia. *Tropilaelaps* mites are also known vectors of honey bee viruses. Hence, mite load may also affect the virus levels and the immune responses of infested bees. In this study, the mRNA expression levels of four antimicrobial peptide encoding genes (*abaecin*, *apidaecin*, *eater* and *hymenoptaecin*) were measured in honey bee pupa challenged with different number of inoculum *Tropilaelaps* mites. All samples were measured for the amount of Acute bee paralysis virus (ABPV), Kashmir bee virus (KBV), Sacbrood virus (SBV), Deformed wing virus (DWV) and Black queen cell virus (BQCV). Our result showed that *apidaecin* was significantly down-regulated when cell cappings of newly sealed larvae were opened and closed without mite inoculation (o/c) and in pupae infested with 1-2 mites as compared to the control (undisturbed brood, no mite inoculation). However, an up-regulation of *apidaecin* and *eater* genes was observed in pupae with higher number of infesting mites when compared to the o/c group. The infested pupae were infected with variable levels of DWV and BQCV (86.7% and 83.3%, respectively). However, the presence of these two honey bee viruses was not related to the number of *T. mercedesae* infecting the pupae and did not alter the targeted immune genes. Thus, from our observation, the expression levels of *apidaecin* could be affected by *Tropilaelaps* mite infestation.

The physiological pathways elicited in response to a *Nosema ceranae* infection

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Nosema ceranae is a widespread, relatively new, microsporidian gut parasite of the western honeybee and is implicated to play a role in the recent decline of honeybee health. However, the actual impact on honeybee fitness and the virulence of this parasite has been hard to pinpoint because it is a chronic infection with relatively benign pathological effects. Energetic stress has emerged to be a potential driver of the physiological changes which could cause the accelerated behavioral maturation observed in infected bees. This is not surprising given that *N. ceranae* requires energy from its host to reproduce and disrupts the honeybee gut lining which can lead to a lack of nutrient absorption. In response to energetic stress, insects have been shown to release both adipokinetic hormone to mobilize fat stores and octopamine to increase searching activity for food. To determine whether the behavioral changes observed in infected bees is from *N. ceranae* itself or the energetic stress it causes, gene expression of both hormones and their receptors was measured in infected and uninfected bees that were starved and satiated (N = 40). The results pre-

sented here will disentangle if energetic stress itself is the cause of the behavioral effects observed from a *N. ceranae* infection, which has been shown to reduce the honeybee life-span in half, and will also determine whether or not the honeybee can compensate for this energetic stress. In light of these results, considering energetic stress as a common underlying mechanism for pollinator decline will also be discussed.

Bee Products

Organizers: Francisco Tomás Barberán, Vassya Bankova

Bee products include economically important foods and materials. The products in the market originate mainly from honey bee although the products from stingless bees are gaining more relevance in the international trade.

Bee products include honey (unifloral and multifloral and honeydew), meads (fermented honey drinks), bee pollen, beeswax, propolis, royal jelly and bee venom. The knowledge in their chemical composition, biological effects and health applications of these products have advanced substantially during the last years, and this will be reviewed in the present symposium. These bee products are a consequence of the interaction of the bee with the plants they visit for feeding and collecting materials needed for the life in the hive. This interaction is still poorly understood and advances in this area will be reviewed.

Invited speakers: M. Teresa Sancho, Miguel Vilas-Boas

GC-MS as a dereplication strategy for propolis chemical type

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The rapid identification of known secondary metabolites from chemical profiling of plants and other natural sources, known as dereplication, is gaining importance for phytochemistry. GC-EIMS is a powerful analytical platform for dereplication, combining the unprecedented resolving power of capillary GC with the structural information provided by EI mass spectra and supported by rich spectral libraries.

Propolis composition is highly variable and depends on the specificity of the local vegetation; bees choose defensive bioactive plant materials as resin source to produce propolis. Thus, the finding of new types of propolis from unexplored regions has the potential to uncover new biologically active compounds with important pharmacological effects. For this reason, beginning the study of propolis from unstudied regions, it is important to find out if it belongs to an already known propolis type. GC-MS of silylated ethanol extract of propolis is an excellent tool for propolis chemical type dereplication. The most important and characteristic constituents of the known propolis types with their EI mass spectra will be overviewed. This approach to characterization of propolis samples by a comprehensive GC-MS chemical profiling gives also possibilities to uncover some novel compounds by dereplication studies for targeted isolation, as demonstrated by the cases of propolis from Oman and Malta.

Antibacterial activity and antibiotic-enhancing effects of honeybee venom against methicillin-resistant *Staphylococcus aureus*

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Methicillin-resistant *Staphylococcus aureus* (MRSA), along with other antibiotic resistant bacteria, has become a significant social and clinical problem. Here we assessed the in vitro activities of bee venom (BV), alone or in combination with ampicillin or penicillin on growth of MRSA strains. The minimum inhibitory concentrations (MIC) of BV were 0.085 g/ml and 0.11 g/ml against MRSA CCARM 3366 and MRSA CCARM 3708, respectively. The minimum bactericidal concentrations (MBC) of BV against MRSA 3366 was 0.106 g/ml and that against MRSA 3708 was 0.14 g/ml. The bactericidal activity of BV corresponded to a decrease of at least 3 log cell forming unit(CFU)/g cells. The combination of BV with ampicillin or penicillin yielded an inhibitory concentration index ranging from 0.631 to 1.002, indicating a partial synergistic effect. The expression of *atl* gene was increased in MRSA 3366 treated with BV. These results suggest that BV exhibited antibac-

terial activity and antibiotic-enhancing effects against MRSA strains.

Using GIS to find suitable areas for organic beekeeping

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The EU regulation on organic production has specific requirements for beekeeping. The landscape must allow for the bees to gather nectar and pollen essentially from sources of organic crops, spontaneous plants or plants treated with low environmental impact methods. To secure these conditions an area of 3 km radius around the apiary, a total of approximately 2800 ha, must be certified. According to the Regulations article 13.2, the Member States may designate the area available to organic beekeeping.

The land use in Denmark is characterised by agriculture. Approximately 60% of the total area is arable. Organic production in Denmark is registered on approximately 180000 ha, or about 7% of the total agricultural area. While it may seem easy to find areas of 2800 ha with space for apiaries, within the total of 180000 ha, the very patchy distribution of the landscape makes this a fairly complex task, beyond the capacity of most beekeepers.

The application of GIS to the designation of areas for organic beekeeping will be demonstrated. We have produced detailed maps for organic beekeeping in Denmark, based on the EU regulation. For Denmark, the organic beekeeping will mainly be confined to areas of natural vegetation. Due to the widespread conventional agricultural farms, most organic beekeeping areas are found in coastal regions. Hence the opportunities for large volume production of organic honey are limited in Denmark.

Composition and bioactive properties of Portuguese propolis

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The food and the pharmaceutical industry has encouraged, in recent years, the emergence of countless research works dealing with beehive products, particularly focusing in propolis composition and its biological properties. Propolis is a resin obtained from plants which the bees use to protect the honeycomb against the ingress of air currents, predators and microorganisms and whose chemical composition is highly variable, depending strongly on the plant sources available to the bees at the site of collection.

The aim of this work was to study the Portuguese propolis in order to establish the quality criteria and biological activity that enable the association of this beehive product with its phytogeographic origin, in an attempt to standardize the product and contribute for its commercial valorisation. Propolis from six regions of Portugal, including islands,

was fully characterized in terms of physicochemical properties, HPLC and LC/DAD/ESI-MSⁿ determination of the phenolic composition, GC-MS evaluation of the volatile fraction, and antioxidant activity, enabling the establishment of quality standards. The results allowed the definition of two standards for the Portuguese propolis and the association with its most probable origin, *Populus* spp. e *Cistus ladanifer*. Also, electrochemical methods were applied as a potential tool in the rapid antioxidant activity evaluation. Finally, the antiprotozoal, antibacterial and antifungal activity of some Portuguese propolis samples and its two potential floral sources were screened, with the results revealing a low selectivity and moderate activity against pathogenic protozoa. The best bioactivity results were verified against the protozoa *Trypanosoma brucei*. and the fungus *Trichophyton rubrum*.

Free-choice profile as a promising method to differentiate unifloral honeys

Ana Pascual-Maté, Elena G. Rodríguez-Morales, Miguel A. Fernández-Muiño, M. Teresa Sancho.

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Free-choice profile (FCP) is a sensory analysis procedure, in which untrained consumers, after being provided with adequate instructions, use their own words to describe and quantify the intensity of some sensory attributes of a given product. In this research, free-choice profile was chosen to assess the Spanish consumer acceptability of 9 representative Turkish honeys from different botanical origins, as well as of 1 adulterated sample. The panel of assessors was composed by six females and one male, aged between 21 and 50 years old. Their participation was voluntary and not rewarded. In the first session, assessors received a brief outline of the FCP procedure and were asked to describe the samples in terms of attributes for appearance, odour, flavour and other sensations in their mouth and throat, using their own vocabulary. For the second session, duplicate individual score cards were prepared to evaluate the intensities of each sensory attribute generated during the first session. In the third session, each assessor re-assessed in duplicate each honey, giving a score on all sensory attributes previously described for each sample by all assessors, so that consensus could be achieved. The data acquired by FCP were processed by Generalized Procrustes Analysis (GPA). Despite not being originally aimed at honey characterization, free-choice profile allowed to perfectly classify the samples according to their botanical origins, so that this procedure should be thoroughly studied and optimized in the near future as a promising method to characterize unifloral honeys.

Study of some functional properties of a food product made with honey and propolis

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Nowadays, the consumers are showing great interest in foods with added bioactive compounds potentially healthy and useful to prevent some diseases. This research has been focused on the design of a food product made with honey enriched with propolis, researching total phenolics and flavonoids, as well as such potentially functional properties as TEAC antioxidant capacity and antibacterial activity against *Staphylococcus aureus*. With regard to the design of the food product in order to get a suitable consistency of the propolis extract to be homogenized with honey, different compositions of a hydroalcoholic extracting solution for propolis and times of maceration were studied. Honey was mixed with propolis extracts, and the mixtures were subjected to a subsequent hedonic analysis with a representative number of consumers, in order to choose the most welcome products. In respect of the parameters and properties analyzed, such small quantities as 0.3% and 0.5% of propolis increased about 1.5 times the total phenols content of honey and between 3 and 16 times the total honey flavonoids' amount. The designed products showed significantly higher TEAC antioxidant activities than the honey with no propolis added. According to the data from minimum inhibitory concentrations and minimum bactericidal concentrations, propolis also increased honey bactericidal effect against *Staphylococcus aureus*. In conclusion, products made with honey and a small amount of propolis seem to be promising functional foods regarding some compounds, as well as antioxidant and antibacterial activities.

Advances in Chemical Ecology of Bees

This symposium aims at highlighting recent advances in our understanding of chemical communication in bees. Chemical messages are essential for regulating social interactions within bee species, but are also exploited by predators and parasites. Here we would like to discuss novel data on the production and perception of chemical messages and their role in shaping behavioural interactions.

Organizers: Patrizia d'Ettorre

Invited speakers: Yves Le Conte, Rita Cervo, Thomas Schmitt

Ecology and evolution of the utilization of resin derived terpenes in stingless bees

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Stingless bees are among other eusocial bees like *Apis* and *Bombus* very important pollinators. They are distributed in tropical and subtropical regions all over the world. In contrast to other eusocial bee species, stingless bees collect not only pollen and nectar but also large amounts of plant derived resin. The sticky resin is used for nest construction and maintenance as well as against potential enemies like ants. The chemistry of the resin is known to be an effective agent against microorganisms and/or fungi. Resin is composed, amongst other substances, of large amounts of various terpenes, which are known for its repellent and toxic effects. However, terpenes are also used by many organisms as infochemicals. Accordingly, we could show that stingless bee workers use mono- and sesquiterpenes to locate required resin resources. Surprisingly though, a large number of stingless bee species are able to extract specific terpenes from resin to incorporate these substances into their cuticular chemistry. We could demonstrate that individuals from different species extract different terpenes from the same resource and thus show a species-specific cuticular profile dominated by terpenes. We are currently working on the intra- and inter-specific functions of these terpenes, particularly their function concerning the behavior and the ecology of stingless bees. Finally, we are also interested in the biogeography and the evolutionary history of the ability to acquire terpenes from the environment.

Chemical detection of threats at the hive entrance: testing for recognition of quantitatively and qualitatively chemical profile alteration in *Apis mellifera*

Rita Cervo, Federico Cappa, Claudia Bruschini, Stefania Meconcelli, Ilaria Protti, Giuseppe Pieraccini, Stefano Turillazzi

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Varroa destructor mite represents a crucial threat for the extensive worldwide beehive losses. Therefore, effective methods to stop mite transmission among beehives are fundamental to save honeybee colonies. Nevertheless, the strategies adopted by mites to invade a new colony and the potential counter-adaptions of honeybees to defend their hives from outer threats are poorly investigated.

We showed that a change in the cuticular hydrocarbons (CHCs) signatures of bees from highly infested colonies promotes mite departure from exploited hives by riding pollen foragers. Once on a forager, parasites may quantitatively and/or qualitatively alter the chemical profile of their hosts to make them undetectable to promote the mite entrance in other colonies.

We investigated the existence of a quantitative threshold for CHCs perception in

honeybees by presenting different fractions of a foreign forager's chemical profile to honeybees at the hive entrance. We found that, in honeybees, discrimination occurs only above a certain amount of cues, suggesting that parasite/pathogens could induce an undetectable chemical profile in the host to elude the colony defence to favour their own transmission. Moreover, our GC-MS analysis showed qualitatively alterations induced by *Varroa* on the CHCs host profile. By using field bioassays we tested the bees' ability to discriminate individuals with a parasite-altered cuticular profile.

Our results underlined the importance of the quantitative threshold, suggesting possible breaches in the honeybees recognition process but also highlighting the honeybees ability to recognize CHCs host profile alterations induced by the mite that might prevent the entry of parasitized individuals.

Behavioral and Molecular Studies to Enhance *Varroa*-Specific Hygienic Behavior of Honey Bees (*Apis mellifera*)

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Despite the importance of the honey bee (*Apis mellifera*) to global food security and natural ecosystem maintenance, the last six decades have seen significant honey bee declines. These declines are attributed largely to the spread of novel parasites and the pathogens they vector, most notably the ectoparasitic mite *Varroa destructor*, considered by many to be the most important threat to apiculture today. *Varroa* resistance programs have been successful and rely primarily on selection for hygienic behavior. However it remains unclear what mechanisms trigger hygienic removal of *Varroa*-infested brood. We tested the hypothesis that adult hygienic behavior is influenced by breed-specific, damage-dependent brood signals. We cross-fostered mite-infested and control brood among three honey bee breeds to determine the effect of interactions between various adult and brood genotypes with respect to hygienic removal behavior. We then characterized cuticular chemical profiles and quantified Deformed Wing Virus for individual mite-infested and control brood from each the three breeds. Significant effects of brood genotype, hive genotype and mite presence on removal behavior support our hypothesis. Significant effects of age, brood genotype and mite presence on chemical profiles also support our hypothesis, and chemical profiles will be compared with virus and behavioral results to identify specific cuticular chemicals associated with disease and removal behavior. By improving understanding of and capacity to enhance mite resistance in honey bees, this study may lead to improved *Varroa* resistance by facilitating development of novel bioassays for selective breeding of mite-specific removal.

Honeybee larval food and Varroa biology

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The composition of the foodstuff provided to the growing larvae by nurse bees has important implications for the life of the honeybee colony. However, larval food seems to play a crucial role also in the intricate relationship between the honeybee and its major parasite: the mite *Varroa destructor*. In particular, it has been shown that a substance from larval food can attract the mite inside the brood cell being therefore involved in a critical stage of mite's life cycle, that is the beginning of the reproductive phase. Furthermore, another compound from royal jelly seems to repel the mite, accounting for the observed reduced invasion of queen cells.

This information will be put in the context of the available data on semiochemicals involved in the process of cell invasion by the mite and commented in the framework of the current knowledge on chemical communication strategies in host-parasite interactions.

Experimental evidence that in *Melipona subnitida* (Apidae, Meliponini) the queen's egg triggers cell sealing

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The capping of brood cells in meliponine bees is nearly universal and probably essential to confront reproductive workers in their pursuit of usurping brood cells. Cell capping or sealing is done by a single worker and happens swiftly just after a queen has deposited her egg on top of the larval fluid. In this study, in three colonies it was experimentally determined whether it is either the egg or the upper cell rim, on which the queen positions herself during egg laying, or both, that trigger cell sealing. Thereto, recently deposited queen's egg were collected and put in ready but still empty brood cells and operculating was observed. Parts of the upper cell rim that had been in contact with the queen's abdomen during oviposition were collected and handled likewise. Next, the findings were underpinned by chemical analyses of egg's surfaces and upper cell rim parts, and verified by the execution of bioassays. By perforating cell caps one hour after sealing, the lasting of the cell-sealing triggering signal was determined. Results revealed that a triggering, volatile substance is present on the queen's egg, likely not on the upper cell rim, and that it persists for at least one hour. The triggering signal is intra-specific because a queen egg from one colony can set off sealing in another. Our tests furthermore showed that there is no need for the physical presence of an egg for a cell to be closed, nor needs a cell rim to be smooth.

Bee health and chemical communication in honey bee

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The honeybee colonies are fascinating example of social organization based on communication. Chemical communication is particularly important on honeybee colony social regulation and pheromones produced by different actors of the colonies, the queen, workers and brood, had been shown to be keys in this mechanisms.

Massive honeybees losses have been reported in the world, and the specific causes are usually unknown. Single factors have not yet explained this global decline, leading to the hypothesis of multifactorial syndromes. Consequently, testing the integrative effects of more than one stress is an interesting approach to understand colony losses.

One hypothesis would be that stresses related to honeybee losses would act on chemical communication processes, modulating production or reception of the pheromonal compounds.

We tested the effects of an infectious organism and an insecticide on honeybee pheromonal communication. We demonstrated a synergistic effect between both agents significantly weakening honeybees. *Nosema* in combination with imidacloprid caused a higher rate of mortality. *Nosema* changed worker pheromone production which shows that a pheromonal disruption related to different stresses could be involved in weakening colonies. Data will also be presented on queen pheromonal production.

Varroa destructor can also disrupt chemical communication between individuals of the colony, but we will present evidence of chemical communication involved in the recognition of the mite by the worker bees at the behavioral and molecular level.

We, thus, provide evidence for integrative effects of different agents and stresses on honeybee health modulating chemical communication in the honeybee.

Haplotype determination of *Varroa destructor* mites in Serbia using ARMS and RFLP methods

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Varroa destructor mite, represented by Korea (K) and Japan (J) mitochondrial haplotypes, is considered to be the main cause of winter losses of *A. mellifera* colonies worldwide. However, in recent study conducted in Serbia, single nucleotide polymorphisms (SNPs) was detected within cytochrome oxidase 1 (*cox1*) and cytochrome b (*cytb*) mitochondrial DNA (mtDNA) sequences, defining Serbia 1 (S1) and Peshter 1 (P1) haplotypes, respectively.

In this study, 89 females of *V. destructor* were used for haplotype determination.

Samples originated from three localities in Serbia where S1 and P1 haplotypes were already affirmed in our previous study. Determination of *cox1* and *cytb* sequences from analyzed individuals was done using ARMS (Amplification Refractory Mutation System) PCR and RFLP method based on *AseI* restriction enzyme, respectively.

The S1 haplotype of *V. destructor* was detected in 32 samples and at all localities. RFLP pattern typical for P1 haplotype was observed in 14 samples, while 43 samples belonged to the K haplotype. There were no samples with both S1 and P1 pattern.

This preliminary study indicates the usefulness of ARMS PCR and RFLP method in determination of newly described *V. destructor* haplotypes. The virulence of detected haplotypes and their possible specific traits concerning host-parasite relationship remain to be examined.

Genomics

Organizers: Michael Lattorff, Silvio Erler

Since the release of the honeybee genome sequence back in 2006 many other species of bees have entered the genomic era. Several honeybee species, bumblebees and primitively eusocial bees are being sequenced for full genomes, EST libraries or transcriptomes. With the decreasing costs and further technological developments, genomic methods will be applied to answer a diverse array of questions in more and more species. This opens the road for large-scale studies in population genomics, comparative genomics, molecular evolution and functional studies that will help in understanding how sociality interacts and shapes genomes.

With the help of genomic techniques we are now able to get a deeper understanding of processes like caste determination and differentiation, one of the basic features of eusociality. We are able to infer the selective pressures in relation to sociality, either based on evolutionary rates of genes or by comparative analyses of gain and loss of genes or groups of genes (e.g. immune genes).

Fundamental processes can now be studied in detail allowing us to functionally analyse the complex regulatory mechanisms of division of labour. Interactions between individuals of colonies, which produce the colony phenotype, can be broken down to the molecular level as well as the interactions of bees with their environment. Since there are serious reports about pollinator declines and colony collapse disorder (CCD) and increased winter mortality in honeybees, many new insights on these threats have been gained from studies utilizing genomic techniques.

This symposium aims at integrating the many different contexts in which genomic techniques are applied to bees. These range from applied aspects, like bee breeding, to the insight into one of the major transitions in evolution – from solitary to social living organisms

Invited speakers: Robert Paxton, Matthew Webster

Synthesis of transcriptome data to explore honey bee-pathogen molecular interactions

Vincent Doublet, Yvonne Poeschl, Andreas Gogol-Döring, Christina M. Grozinger, Robert J. Paxton and members of the TransBee workshops

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Pathogens and parasites are arguably the most important cause of honey bee losses, threatening the ecosystem service of pollination. Host-pathogen relations are underpinned by intimate molecular interactions. Disparate datasets have been generated on the transcriptome response of the honey bee to pathogens. To explore the commonality in host responses to pathogen challenge, we coordinated two ‘transbee’ workshops funded by the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig (www.idiv-biodiversity.de) in which we synthesized current microarray and next-generation sequence data of 18 published and non-published transcriptome datasets, generated after infection by gut pathogens *Nosema* spp., viruses, or the parasitic mite *Varroa destructor*. In order to compare across studies, all genes were ranked on their differential expression level between control and treated honey bees in all datasets, and a rank product analysis was performed across them. Although considerable variation was observed across studies, a specific response of honey bees to *Nosema* spp., viruses and *V. destructor* mites was apparent. As expected, immune genes were largely represented within the most regulated genes. We also present other candidate genes, gene networks and metabolic pathways included in the response of honey bees to pathogens and parasites. With ca. 20 additional colleagues, we refine our analyses to identify those molecular pathways that are potentially involved in honey bee loss.

A worldwide survey of genome sequence variation reveals the evolutionary history of the honeybee *Apis mellifera*

Andreas Wallberg, Fan Han, Gustaf Wellhagen, Bjørn Dahle, Masakado Kawata, Nizar Haddad, Zilá Luz Paulino Simões, Mike H. Allsopp, Irfan Kandemir, Pilar De la Rúa, Christian W. Pirk, Matthew T. Webster

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The honeybee *Apis mellifera* has huge ecological and economic importance, yet populations are declining while studies of genetic variation remain limited. Here we analyse 140 honeybee genomes from a worldwide sample to illuminate the evolutionary history and genetic basis of local adaptation of this species. We find evidence that population sizes have fluctuated greatly in the past, mirroring historical fluctuations in climate, although contemporary populations have high genetic diversity, indicating the absence of domestication bottlenecks. Levels of genetic variation are strongly shaped by natural selection and are highly correlated with patterns of gene expression and DNA methylation. We identify hundreds of genetic variants involved in local adaptation, which are enriched in

genes expressed in workers and in immune and sperm motility genes, which may underlie geographic variation in reproduction, dispersal and disease resistance. This study provides a framework for future investigations into responses to pathogens and climate change in honeybees.

Identification and recombinant production of giant Asian honeybee silk proteins

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Silk, a classic biopolymer that produced by numerous insects. The most wellknown silk are from silkworm and spider dragline that has been used for a century. Unlike ancient silk, silks derived from honeybee, ant and hornet are small and non-repetitive sequences. In the present contribution, we identified four silk fibroins genes from the open-air nesting giant Asian honeybee (*Apis dorsata*)(*AdorF1-4*). It was found that *A. dorsata* silk proteins are ~30 kDa in size, rich in alanine (>30%). Moreover, the silk genes were successfully cloned and expressed by *Escherichia coli*. The recombinant silk proteins were purified, re-folded and generated silk films. According to the circular dichorism results demonstrated that recombinant silk proteins are formed coiled-coil structure. The silk films generated by solvent-casting technique, the clear and elastic-film were obtained. The silk film properties were assessed.

Polymorphism for the type of parthenogenesis in laying workers of *Apis mellifera capensis*

Denise Aumer, Antje Jarosch-Perlow, Mike H. Allsopp, Robin F.A. Moritz

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Normally honeybee workers refrain from reproduction, but if they lay eggs they produce male offspring by arrhenotokous parthenogenesis. An exception are laying workers of the Cape honeybee *Apis mellifera capensis*. Thelytokous parthenogenesis, the production of female offspring, has been claimed to be a characteristic trait of *A. m. capensis* workers. We sampled workers from two wild colonies of *A. m. capensis* at Cape Point Nature Reserve as far away as possible from the hybrid zone with the adjacent subspecies *A. m. scutellata* in the North. We individually tested the mode of parthenogenesis of these workers by letting them produce offspring and genotyping both mother worker and offspring. We found the mode of parthenogenesis to be highly polymorphic with an equal frequency of thelytokous and arrhenotokous laying workers. We found no evidence for amphitoky (a single worker produces both male and female offspring). Previous claims that

laying workers of *A. m. capensis* are exclusively thelytokous may have resulted from the strong intracolony selection in favour of thelytokous workers, which can pheromonally suppress reproduction of the arrhenotokous workers in the colony.

Neural Basis of Behaviour in the Honeybee

Organizers: Wolfgang Blenau, Geraldine A. Wright

The honeybee - a pollinator extremely important for agriculture and as a producer of honey - performs complex behaviours including learning, communication, and navigation with a brain of less than 1 million neurons. For example, honeybees use the dance language to communicate distance and location and reward value of floral resources. This is the only known non-primate symbolic language and its discovery won Karl von Frisch the Nobel Prize in 1973. Because of the honeybee exhibits impressive cognitive capabilities achieved with a small brain, it has become an important model organism for understanding the neural basis of behaviour.

Another - rather unpleasant - reason for intensification of neurobiological research in bees is the increasing number of colony losses in the past 15 years. Among other proposed causative agents, sub-lethal effects of pesticides may contribute to this phenomenon, though the mechanisms are largely unknown. Most of the substances under suspicion target proteins in the central nervous system (e.g., neonicotinoids nicotinic acetylcholine receptors). Thus, an increasing number of researchers are investigating the sub-lethal effects of these substances on honeybee behaviour (learning, navigation), neuronal physiology, and development.

In this symposium we wish to provide a forum for the discussion of cutting edge research on honeybee neurobiology and applied research regarding the neurophysiological effects of pesticides on the bee brain.

Invited speakers: Keram Pfeiffer, Markus Thamm, Mary J. Palmer

PKG in honeybees: From gene expression to behavior

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Honeybees exhibit a fascinating division of labor among worker bees, displayed by performing different tasks as a function of age. The most distinct tasks are providing food for the brood (nursing) and collecting pollen and nectar (foraging). In addition, nurse bees and foragers differ in their sensory responsiveness, for example to gustatory stimuli. The last years have shown that nutrition-associated signaling pathways are important regulators of age-dependent division of labor. A number of experiments suggest that the cGMP/PKG pathway is an important player in food-searching related behaviors. Our experiments in honeybees demonstrate that pharmacological activation of PKG increases sucrose responsiveness of nurse bees to the level of foragers.

One honeybee PKG is encoded by the *foraging* gene (*Amfor*). This highly conserved gene is the center of our investigation. Our analysis reveals the presence of alternative splicing of this gene. One isoform is expressed in the brain. Division of labor and sucrose responsiveness in honeybees correlate with *foraging* gene expression in distinct brain regions. We developed a honeybee-specific antibody against the PKG and show PKG-like expression in the mushroom bodies, the subesophageal ganglion, and the *corpora allata*. These brain structures are involved in the processing of olfactory, gustatory, and hormonal signals. Our experiments imply an important role of PKG in honeybee division of labor through mediating nutrition-associated signals.

Investigating the synaptic basis of sensory processing in honeybee Kenyon cells.

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In insects, higher cognitive functions such as associative learning are dependent on the mushroom bodies (MBs), which are particularly large in social insects such as honeybees. In the MB calyces, Kenyon cells (KCs) receive excitatory input from sensory projection neurons via nicotinic ACh receptors (nAChRs), inhibitory input from MB feedback neurons via GABA receptors, and modulatory input from distributed neurons that release biogenic amines such as octopamine. However, the synaptic mechanisms underlying sensory processing in the MB calyces are not well understood. To examine the properties of endogenous synaptic input to KCs, we are using whole-cell recordings from KCs in acutely-isolated honeybee brain. The majority of KCs exhibit nAChR-mediated spontaneous EPSCs (sEPSCs) with fast kinetics, whereas a minority show an additional sEPSC subtype with slow kinetics. Similarly, some KCs exhibit ACh-evoked somatic responses with distinct fast and slow components. The octopamine receptor antagonist epinastine inhibits both ACh-evoked responses and the fast sEPSCs in KCs, but does not inhibit GABA-

evoked responses. KCs exhibit a spontaneous GABA receptor-mediated tonic current that is inhibited by picrotoxin but not bicuculline. The underlying receptors have an estimated single-channel conductance of 27 ± 3 pS, similar to that of RDL receptors. The tonic GABA receptor conductance acts to reduce neuronal excitability, a property that is likely to contribute to sparse coding of sensory information in KCs. Thus, whole-cell recordings from KCs in intact MBs can be used to elucidate the neurophysiological basis of sensory processing and synaptic mechanisms underlying associative learning in honeybees.

A potential site of time-compensation in the polarization vision pathway of the honeybee

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Honeybees possess a time-compensated sky compass which enables them to infer a fixed earthbound direction, e.g., to a food source or their hive, from the continually changing solar azimuth. In addition to direct sunlight bees can use the sky polarization pattern as an orientation cue. Experiments over more than six decades provided insight into many aspects of this fascinating behavior, but the underlying neuronal mechanisms are still elusive.

Polarized light is perceived by a highly specialized dorsal rim area (DRA) of the compound eye. Using dye injections, we could trace a pathway from the DRA via the dorsal medulla and the lower subunit of the anterior optic tubercle to the bulbs of the median protocerebrum. Here neurons form large synaptic complexes of up to 7 μ m diameter with GABAergic neurons of the lower division of the central body. Cholinesterase activity labeling suggests that these microglomerular synapses are cholinergic (Kreissl and Bicker 1989, *J Comp Neurol* 286: 71-84), which could potentially make the sky-compass system vulnerable to neonicotinoids.

Time compensation of a sky-compass system requires that information about the solar azimuth is integrated with circadian information. To reveal potential sites of time-compensation within the brain, we combined dye injection experiments with anti-PDH immunostaining. Anti-PDH labels pigment dispersing factor, an output signal of the insect circadian clock. In the medulla, we found neurons of the polarization-vision pathway to branch in the same layer and in close proximity to PDH-ir neurons. This suggests that the medulla of honeybees is a site of time-compensation.

Function and distribution of 5-HT₂ receptors in the honeybee (*Apis mellifera*)

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Serotonin plays a pivotal role in regulating and modulating physiological and behavioral processes in both vertebrates and invertebrates. In the honeybee (*Apis mellifera*), serotonin has been implicated in division of labor, visual processing, and learning processes. Here, we present the cloning, heterologous expression, and functional and pharmacological characterization of two honeybee 5-HT₂ receptors, Am5-HT₂ α and Am5-HT₂ β . Each receptor gene also gives rise to alternatively spliced mRNAs that possibly code for truncated receptors. Only activation of the full-length receptors with serotonin caused an increase in the intracellular Ca²⁺ concentration. The effect was mimicked by the agonists 5-methoxytryptamine and 8-OH-DPAT at low micromolar concentrations. Receptor activities were blocked by established 5-HT receptor antagonists.

In the brain, the expression of both 5-HT₂ receptors is low compared to other 5-HT receptor subtypes. In contrast, high transcript numbers were detected in exocrine glands suggesting that 5-HT₂ receptors participate in secretory processes in the honeybee. Interestingly, the expression of the full-length Am5-HT₂ α transcript was higher than that of the shortened splice variant, whereas the opposite holds true for Am5-HT₂ β . Age-dependent division of labor in honeybees is correlated with increasing serotonin levels in the brain. Therefore, we compared 5-HT₂ receptor expression in the brains of workers of different age groups, and daily oscillations in 5-HT₂ receptor expression. In conclusion, this study marks the first molecular and pharmacological characterization of two 5-HT₂ receptor subtypes in the same insect species. The results presented should facilitate further attempts to unravel central and peripheral effects of serotonin mediated by these receptors.

Posters

Advancing Bee Health

A survey of honey bee pathogens in Algeria (in 2013)

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Honey bee colony collapse syndrome is a sanitary and ecological worldwide problem. The aim of investigations was the identification of pathogens in samples of honeybee sent from apiaries where mass losses of bee colonies were observed. 167 bee samples were collected from Algerian apiaries that displayed severe losses and mortality during February 2013 up to June 2013. The presence of *Nosema apis* and *ceranae*, *Varroa destructor*, *Acarapis woodi*, *Ascosphaera apis* and *Peenibacillus larvae ssp. larvae* were analysed using standard OIE methods.

Our studies indicate that *Nosema ceranae* is the dominant species in Algeria with an estimated 54 % of hives infected. *Nosema apis* infections were only observed at very low levels (1 %), and occurred only as co-infections with *Nosema ceranae*. Spores of *Peenibacillus larvae* were detected in 39 % of the apiaries. The tracheal mite, *Acarapis woodi*, was detected in one *Apis mellifera intermissa* colony. The infestation levels *Ascosphaera apis* 7 %. All colonies infested by *Varroa*, over 65 % of hives are characterized by an infestation rate of more than 10 % of *varroa* mites on bees. The presence of 2 pathogens (*Varroa* and *Nosema*) were simultaneously detected in 41 % of honeybee colonies. The presence of 3 pathogens (*varroa*, *Nosema* and *Peenibacillus larvae*) were simultaneously detected in 29 % of honeybee colonies.

Our data suggest that in our country, *Nosema ceranae*, *Varroa destructor* and *Peenibacillus larvae* are the pathogens more frequently found and probably the key factors in the colony losses phenomenon.

Holistic screening of collapsing honey bee colonies in Spain: a case study

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In recent years, an increase in honey bee colonies losses has been reported in many European and North American countries. Although multiple factors appear to be involved, pathogens may have a particularly important influence on this decline. In the present study we present a holistic screening of three professional apiaries in Spain with a high level of colony losses. Colonies were tested for the presence of multiple drivers including patho-

gens, pesticides and foraging flora, but only a few of them could be detected. Omnipresent were *Nosema ceranae* and Lake Sinai Virus, a moderate prevalence of Black Queen Cell Virus and Trypanosomatids was found, whereas Deformed Wing Virus, Aphid Lethal Paralysis Virus and Neogregarines were diagnosed in just one or two colonies. The palinologic study demonstrated that all colonies were foraging on wild vegetation and the pesticide residue analysis was negative for neonicotinoids. The evaluation of the putative role of each of these drivers in the depopulation process proved that they might differ among geographic regions with different environmental conditions, beekeeping and agricultural practices.

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Immune response in nurses and forager bees (*Apis mellifera*) after artificial immune challenge

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The immune system protects animals against internal and external stressors. The innate immune response is shared by many animal phyla, and is known to decline during aging. The honey bee, with its flexible aging patterns, offers the opportunity to study flexible aging of the immune system, including the reversal of immunosenescence. In honey bee, as in other insects, the key immune regulators are fat body and the circulating hemocytes (blood cells). The fat body is a production site of effector molecules, while hemocytes are involved in phagocytosis, melanization and encapsulation. The present study investigated immune response in foragers and nurses to test for social task dependent response patterns, independent of chronological age.

Nurses and foragers of the same chronological age (20-25 days) were divided into three groups, one group injected in the abdomen with lipopolysaccharide (LPS), and the second group with peptidoglycan (PGN). In both cases this was done to induce immune response. Third group was injected with phosphate buffered saline (PBS) only and used as a control.

No treatment specific effect was detected. Nurses survived longer than the foragers. Specifically, both nurses and foragers showed similar survival patterns when treated with LPS, PGN or PBS. This could indicate that the damage and inflammatory responses are more harmful than infection responses due to bacterial cell wall components. The treatments gave also different transcriptional patterns of immune related genes; interestingly this pattern differed between nurses and foragers.

Diffusion of *Vespa velutina* in Italy

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Vespa velutina Lepeletier 1836 was detected in France in 2005 in the area of Bordeaux and since its arrival it has spread in a great part of the country and in neighbouring countries. A monitoring activity for intercepting *V. velutina* has started since 2007 in the Italian regions of Piedmont and Liguria which border on France to the West. The main communication routes that join Italy and France, and freight traffic patterns were taken into account. Bottle traps baited with lager beer were used and the trapped insects were removed and determined weekly throughout the active season. The first *V. velutina* specimen, a male, was trapped at Loano (Savona Province, Liguria Region) on 19th November 2012. As a consequence of this catching, the monitoring activity continued in 2013, focusing on the Ligurian coast, and the local beekeeper associations were alarmed. These activities permitted to ascertain the presence of yellow-legged hornet workers in several localities in the provinces of Imperia (west Liguria) and Cuneo (southern Piedmont); active nests of *V. velutina* were also detected and destroyed in some of such localities. The further diffusion of *V. velutina* in Italy is presently monitored in a larger part of the country.

An active surveillance protocol of the main pathogens affecting honey bees in Spain

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The beekeeping sector is suffering unexpected losses in many countries in the last decade. We have developed an active surveillance protocol to monitor the evolution of the prevalence of major pathogens that might be involved in the phenomenon of collapsing honey bee colonies. This active surveillance consists of two cross-sectional systematic apiaries sampling conducted during 2010 and 2011. In each apiary analyzed, six colonies of honey bees were sampled randomly. Each sample was composed of adult worker bees and brood, stored pollen, honey and debris from the bottom of the hive. In total 360 samples were collected in 2010 and 275 samples in 2011. The prevalences obtained for pathogens of adult worker honey bees are (the first number shows the prevalence of 2010 and the second by 2011): *Acarapis woodi* 8.3% and 4%, *Neogregarines* 15.55% and 20%, *Trypanosomatids* 68.8% and 38.18%, *Nosema apis* 15.27% (in both years), *Nosema ceranae* 73.3% and 70.9%, *Varroa destructor* 45.83% and 53.81%. For the pathogens affecting brood the prevalences are: *Ascosphaera apis* 5.27% and 4%, *Melissococcus plutonius* 0.83% and 3.27%, *Paenibacillus larvae* 1.38% and 1.09%. Also, we have analyzed the main virus infecting adult worker bees. As expected *Nosema ceranae* is the most prevalent pathogen,

followed by *Varroa destructor* and their related virus (BQCV and DWV). It has been surprisingly the high prevalence of *Trypanosomatids* found.

Nor Small Hive Beetle or *Tropilaelaps* were detected during the both studied years.

In stored pollen, only 21% of the samples analyzed were positive for a pesticide residue. Acaricides used for control of *Varroa* were the most abundant.

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Passive surveillance of honey bee diseases in Spain

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We have developed a passive surveillance protocol to monitor the evolution of the prevalence of major pathogens in the samples voluntarily sent by beekeepers or veterinary services to the Pathology Laboratory of the Regional Apiculture Center. It is running together with an active surveillance and both might determine the causes involved in the phenomenon of the collapse of honey bee colonies.

In this passive surveillance, the goal was to know the evolution of the diseases and to develop prevention plans quickly and efficiently. Also, it should allow us to an early detection of exotic pathogens such as the small hive beetle (SHB) or *Tropilaelaps sp.*, in case of their entry in Spain.

A total of 204 samples were analyzed over two years (132 in 2010 and 72 in 2011). The results of prevalence in 2010 were: *Acarapis woodi* 2.27%, *Apicystis bombi* 6.81%, *Crithidia mellificae* 15.15%, *Nosema apis* 11.36%, *Nosema ceranae* 56.06%, *Varroa destructor* 12%, *Ascosphaera apis* 32.35%, *Paenibacillus larvae* 11.76%. *Melissococcus plutonius* was no detected.

For 2011 the results were: *A. bombi* 2.77%, *C. mellificae* 12.5%, *N. apis* 4.16%, *N. ceranae* 33.3%, *V. destructor* 18.05%, *A. apis* 30.95%, *P. larvae* 14.28%; *A. woodi* or *M. plutonius* were no detected.

Unfortunately, virus analysis could not be performed because the RNA integrity once the sample get to the laboratory did not allow it.

SHB or *Tropilaelaps* were never detected during the both studied years.

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Essentials oils from Portuguese aromatic flora as a potential alternative for *Varroa destructor* control

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The hemophagous mite *Varroa destructor* Anderson & Trueman is a honey bee colonies parasite, nowadays considered the greatest threat for apiculture worldwide. It was recently regarded, with the viruses they vector, as a possible cause of Colony Collapse Disorder. Besides ineffectiveness, the regular use of chemical treatments accelerated mite population resistance or tolerance development, added by high costs and labour, and residues accumulation in apiculture products. Aromatic plants products, mainly their essential oils (EOs) and components, offer an attractive alternative to synthetic acaricides for *Varroa* control.

EOs isolated from Apiaceae, Asteraceae, Lamiaceae, Myrtaceae and Poaceae species from the Portuguese flora were assessed for acaricidal activity. The EOs were isolated by hydrodistillation and analyzed by GC e GC-MS. The EOs effect on varroa and on the honey bees was evaluated by the complete exposure method, using both varroa-parasitized newly emergent bees and adult bees. Different EOs concentrations were tested, with five replicates *per* experiment (n = 25 bees/tested concentration). Blank, negative and positive controls were included. *In vivo* bioassays were done under controlled temperature and RH, and mites mortality and the toxicity effects on bees assessed after 6, 12, 24 and 48h.

Some EOs showed repellent and/or lethal effect on mites. Running experiments evaluate the EOs concentrations with less toxic effects on the bees and the minimum lethal dose for *Varroa*.

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Holistic evaluation of risk factors in honey bees and wild pollinators. The situation in Spain.

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Various factors have been identified in causing the reduction in pollinators including an expansion of pathogens, the incorrect use of phytosanitary products and/or environ-

mental contaminants, along with other factors such as loss or fragmentation of habitat, invasive species and/or climate change. The current challenge is to consolidate research on the issue using risk weighting, and the evaluation of potential convergence between the key factors that affect the abundance and diversity of the pollinators. Furthermore, we need to advance scientific knowledge regarding the influence of these factors on pollinator health, under realistic field conditions, and particularly bearing in mind our country's climatic conditions. In response to this issue, the focus will be on interdisciplinary exchange and cooperation between academia and industry, within a study development framework, under field conditions and with sampling programs at a regional and national scale. This project will address the following main objectives:

- (a) The evaluation of residue levels, of both pesticides and environmental contaminants on honey bees and wild pollinators,
- (b) The effects of neonicotinoids on the neurological, immune and reproductive systems on honey bees,
- (c) The detection of new *Apis mellifera* pathogens; the evaluation of pathogen interaction,
- (d) A study of the Varroa-virus interaction and its effect on the bee's immune system,
- (e) Evaluation of the effect of harmful factors in the genetic diversity of *Apis mellifera* populations
- (f) Palynological study and a study of the nutritional quality of pollen stored in hives,
- (g) Study of the interrelation between hives, health and environmental conditions,
- (h) Identification of factors and critical correlations, and between factors of concurrent risk,
- (i) Modelling of health and environmental factors.

RésApi, prototype network of apiaries for the diagnosis of colonies before wintering.

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Winter honeybee colony losses are characterized by colonies found dead at the end of winter. Many factors are at issue: honeybee parasites, viruses, pesticides and inadequate diet. Various studies have emerged to understand the origin of these losses. The RésApi project's goal is to understand better winter losses through honeybee colonies' transhumance routes, and their physiologic and sanitary conditions at the entrance of winter.

In three French regions, three beekeepers have been part of this project, informing technicians and scientists about twenty-five hives labelled in two of their apiaries. A total of 18 apiaries and 450 hives are monitored each year. Different types of samples are done all year long to better understand colonies' dynamic and health status:

- land uses of the foraging space and landscape structure are studied for each of the transhumance sites over the whole season ;
- harvest of each hive for each honey flow is recorded by beekeepers ;
- vitellogenin, fat body, viruses and *Nosema* spp. are quantified on bee samples taken at the beginning of winter ;
- varroa mites are counted on bee samples in spring, at the end of summer and at the beginning of winter ;
- beebread is sampled at the end of summer for toxic analysis ;
- colonial parameters are evaluated in spring, at the end of summer and at the beginning of winter.

Data are analysed with a binomial mixed model where colonies are considered as safe (null model) or dead after wintering. Results show the main colonial features characterising safe hives after wintering and address some sanitary conditions related to winter losses.

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The occurrence of external *Acarapis* mites in honey bee colonies in an apiary in Iran

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Acarapis woodi were first described by Rennie as *Tarsonemuswoodi* in 1921. In addition to *A. Woodi*, there are two external mite species in the genus *Acarapis* that infest honey bees.

A. dorsalis and *A. externus* are external parasitic mites that consume the hemolymph of honey bees (Root,1990). These two parasites have been reported in Iran by Mosaddegh & Bahreini in 1994. *A.externus* Morgenthaler is found on the cervix (the neck region) and *A.dorsalis* Morgenthaler is found on the dorsal groove of the thorax (Ibay and Burgett, 1989). Morphologically they are difficult to differentiate from one another and from *A. woodi*, even at high magnification.

About 100 bees were collected from the infested colonies. The sample bees were observed under stereomicroscope with a magnification of 40. High level of external *Acarapis* infestation was detected in the samples. The parasites were seen at different stages of eggs, larvae and adult on the body of dead bees. The adults were moving about on the body of dead bees.

According to the morphological characteristics of the parasite and its location in the body of the bee, the parasite could be *A. externus* or *A.dorsalis* or both. After the study of special characteristics of the parasite *A. externus* was detected.

The main symptoms of the infestation are losses of colonies and the decline of colony

population. They were considered to be harmless by Eckert (1961) and Delfinado-Baker (1984)¹.

The microscopic videos of *Acarapis* will be demonstrated.

Integrated conservation of a rare threatened plant and the community of its wild pollinators: Life+ PP-ICON project (LIFE09/NAT/IT/000212)

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PP-ICON (Plant Pollinator Integrated CONservation approach: a demonstrative proposal) is a 4-year LIFE+ Biodiversity project focusing on the integrated conservation of the locally rare plant *Dictamnus albus* L. and the community of its pollinators.

Actions focus on habitat management, pollinators safeguard, stakeholders awareness and dissemination. Habitat has been restored in order to establish the best environmental conditions for the persistence of the plant. Effective pollinators of dittany have been identified in order to provide them with suitable nesting sites; some bumble bee species have been artificially reared and re-introduced in the area. The maintenance of pollinators is also favoured by the transplanting of autochthonous nectariferous plant species. Through periodic monitoring of plant fitness and pollinator diversity we assess the success of conservation actions.

After three years of project implementation wood management provided positive response, with an increase of incident light and a recovery of flowering. The introduction of artificial nests was successful for a number of solitary bee species (e.g. Megachilidae, *Xylocopa*), while one bumble bee specie (*Bombus terrestris*) was successfully reared in the laboratory and re-introduced in the area. The planted nectariferous plants were effectively visited by wild pollinators. Monitoring data still indicate a general scarcity of pollinating fauna, with a positive relation between pollinator abundance and flowered species diversity.

The dissemination events, focusing on the relationship between plants and pollinators and on the strategies to conserve them, attracted more and more people, contributing to the public awareness on the important theme of biodiversity conservation.

¹ BEEBOOK Volume II , Tracheal mite research

Updating honey bee health status in Ghana: 2013 national survey.

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During the summer of 2013 a survey was conducted through Ghana with the aim to know about the local honey bee health status. No published data was found on databases. Samples from brood, adults and colony products from 45 colonies located in nineteen different locations from all regions of Ghana were obtained. Varroa detection was performed in situ and evidences of any other pest noted on. Samples were taken to Spanish laboratories and analysed by molecular diagnosis to detect pathogens and determine the honey bee race and Varroa haplotype.

Oral data from the questionnaire performed to the beekeepers showed that easily observable pests such as ants, lizards, and even snakes were the biggest problems to beekeepers. Only one out of 42 beekeepers knew Varroa consequences for the colony health.

Results show the presence and distribution of uniquely *Varroa destructor* korea haplotype (Genbank AF106899) through all the regions sampled. The mean prevalence in brood was 15.33% and 0.92% in adult honey bees. *Aethina tumida*, the small hive beetle, was found in almost 50% of all sampled colonies. Molecular diagnosis did not detect any *Nosema* species in samples of adult bees while demonstrated the presence of *Melissococcus plutonius*. Further studies are needed to get a complete overview of the health status of Ghanaian honey bee colonies.

Infection of honeybee larvae by entomopathogenic nematodes – natural model to study honeybee immunity

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Entomopathogenic nematodes (EPNs) of the genera *Heterorhabditis* and *Steinernema* are obligate and lethal insect parasites. In recent years they have been used increasingly as biological control agents. These EPNs are symbiotically associated with bacteria of the genera *Photorhabdus* or *Xenorhabdus*. The bacterial symbionts are essential to kill the host (within 24-48 hours) and digest its tissues to provide nutrients for themselves and for expanding nematodes. We used EPNs infection to study immunity of *Drosophila melanogaster* and here we show for the first time that also honeybee larvae are suitable hosts for nematobacterial complex. We used the tripartite model (honeybee, nematodes, bacteria) to develop standard procedure for testing honeybees' immune response. We optimised the infection for EPN *Heterorhabditis bacteriophora* and *Steinernema feltiae*; both species

cause typical coloration of cadavers (red or yellow, respectively), multiply in honeybee larvae and shows similar mortality with LD50 approx. 5 EPNs/larva. Infection with *H. bacteriophora* can be visualised using GFP labelled symbiotic bacteria or by bioluminescence. Mortality of honeybee larvae was dependent on EPN dose. Higher EPN dose also resulted in higher amount of invaded parasites. Non-segmented larvae are more susceptible to the infection than segmented larvae. We hope that this standardized method can be used as a valuable tool that will help us to describe the overall status of honeybees' immunity.

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Control of *Nosema ceranae* in honeybee colonies using some natural products and antibiotics

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This study was carried out at the apiary of Agricultural Experimental Station, Faculty of Agriculture, Cairo University during spring, 2011. Eighteen honeybee colonies were divided to six groups. In this experiment these groups were treated with Tylosin 1 gm./3 col., Tylosin 2 gm./ 3 col., Flagyl 6 ml. / 4 col. , water extraction of 12 gm. Wormwood / 3 col. These materials were given in one liter of sugar syrup 2:1 where the last group was given the sugar syrup without any additives and left as control. All the mentioned treatments were repeated five times at four days interval to give a satisfactory control. This experiment was conducted during April, 2011. During May, 2011 another eighteen honeybee colonies were divided to six groups. These groups were treated with Flagyl 6 ml./ 3 col., water extraction of 12 gm. of wormwood/ 3 col., Septazole 3 ml./ 3 col. , water extraction of 6 gm. Cinnamon/ 3 col. And the last group was given the sugar syrup as a control. These treatments were applied by the same method as mentioned in the first experiment. All the tested materials gave a small effect in reducing the *Nosema* infection level. After four weeks (Five doses were given), the mean infection levels were 0.82, 0.88, 0.84 0.72, 0.70 and 1.83 X 10⁶ spores/ bee/ col.) in the colonies treated with Septazole 1 ml./col. & 2 ml./col., Flagyl 2ml./col., Cinnamon 2gm./col., wormwood 4 gm./col. And control colonies, respectively.

The safeness and the effects of *Agaricus blazei* Murill extract on honey bee colonies

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This study was done to investigate if supplementation with medicinal mushroom *Agaricus blazei* Murill (AbM) extract exerts beneficial effects on honey bees. Safety of AbM extract, appropriate dose and feeding mode were evaluated in a laboratory experiment on overall 640 bees in 32 single-use plastic rearing cups. Three doses of AbM extract (50, 100 and 150 mg AbM/kg/day) and two feeding modes (sugar syrup and candy) were tested. AbM extract was proven to be safe for the bees in each applied dose. Survival of bees did not depend on feeding mode, but only syrup was fully consumed and ensured the bees to completely intake the given dose of AbM extract. A field experiment was conducted on 26 colonies during three successive years (2010-2013). One autumn and two spring treatments were performed using a dose of 100mg/kg/day only and only with syrup feeding mode. To evaluate the effect of AbM extract treatment, five colony strength parameters were measured: open brood area (OB), sealed brood area (SB), adult bee population (AB), honey reserves (HR) and pollen reserves (PR). The periodical positive effects of AbM extract indicate its potential to enhance colony strength. Brood rearing and adult population growth (OB, SB and AB) were more often significantly increased than honey and pollen reserves (HR and PR). Positive effects were mainly observed during April indicating the benefit of spring treatments. To elucidate the mechanisms underlying the effect of AbM on honey bees, further investigations are necessary.

Development of a new gel product containing formic acid for control of Varroa mite

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Formic acid is used in several methods in the control of varroa, the most important parasite pest of honeybee worldwide. In the present study a new formic acid formulation for varroa mite control has been developed according to European guide lines on veterinary medicines. The formulation has been test under different climatic and regional condition.

The product was developed starting by a gel matrix containing formic acid. Initially, a field test of dose titration was performed to assess efficacy and safety of the formulation. To investigate about efficacy of the treatment and effects on eggs, larvae, adult bee and queens, clinical tests were carried out in several apiaries spread along the Italian territories to evaluate product behaviour in different environmental condition.

The characteristics of the formulation are able to produce a passive evaporation of

formic acid and to guarantee a high safety on the bee colony. During the tests, an average acaricide efficacy rate higher than 95% was observed. At the same time, no adverse reactions were observed on larvae, adult bees and queens comparing with no-treated colonies. An high mortality rate was observed on eggs, but they are quickly replaced by the queens in few days, without consequences for colony survival.

In conclusion, a formulation based on passive evaporation of formic acid related to a gel matrix, supplied in ready to use and safe for user precast pads, allows to control varroa mite infestation, with an acaricide efficacy rate higher than 90%, associated with safety for bee colonies.

Honeybee health in Mongolia

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To better understand the global spread and impact of honey bee pathogens, it seems crucial to study areas, which have received little attention so far. Here we conducted a colony survey in Mongolia, where *Apis mellifera* has been introduced in 1959.

In summer 2013, adult workers were sampled throughout Mongolia (165 colonies, 21 apiaries 9 distant locations). Pooled bees (N=50 from each colony) were analysed with standard PCR methods for infections with black queen cell virus (BQCV), sacbrood virus (SBV), deformed wing virus (DWV), chronic bee paralysis virus (CBPV), Lake Sinai virus (LSV-2), acute bee paralysis virus (ABPV), Israeli acute paralysis virus (IAPV) and Kashmir bee virus (KBV) and the microsporidian *Nosema apis* and *N. ceranae* as well as the trypanosome *Crithidia mellificae*. Standard mite washes were performed for *Varroa destructor*.

Varroa destructor was detected in 8 locations with infestation rates up to 27.3%. *Nosema apis* was not detected and *N. ceranae* was rare (1.5% of the colonies). *Crithidia mellificae* was also uncommon (1.5% of the colonies). The most common virus was SBV (64.2% of colonies); followed by BQCV (48%); DWV (10.5%) and CBPV (5.4%). None of the other viruses was found.

We will present and discuss the phylogenetic relations of these detected viruses in Mongolia with their European and Asian counterparts. Our data suggest that *N. ceranae*, *C. mellificae* and DWV are less common in Mongolia compared to other populations of managed European honey bees in North America and Europe. Potential reasons will be discussed.

The influence of sublethal doses of imidacloprid on protein content and proteolytic activity in honeybees (*Apis mellifera* L.)

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The influence of sublethal doses of imidacloprid on bee protein content and activity of proteinases was analysed. The behavior of bees was also observed and the level of contamination with neonicotinoid in food supplies (collected in bee colonies after feeding) was assessed. The study was conducted in 3 experimental groups, 12 colonies in each group. Bee samples were collected on August 20 and again at the beginning of October 2013. The colonies from groups II and III were contaminated with 5 and 200 ppb of imidacloprid, respectively, group I was control (untreated). Protein concentration in bee tissue extract was analysed with reference to the dose of pesticide and time after contact with this compound. The average activity of proteolytic enzymes was related to the dose of imidacloprid. No differences were found in the pace of feeding on the administered food among the colonies from individual groups. We did not observe differences in the flight activity of foragers or in the pace of taking the administered food neither in control colonies nor in both assay groups. We also did not note differences in wintering, and the wintering colonies in all groups overwintered successfully. Thus, if no symptoms of poisoning are visible in bees, the examination of protein profile can be an excellent indicator of the effect pesticides have on bee health.

Demographics of the European apicultural industry

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Although beekeeping has become an increasingly professional activity in recent years, the beekeeping industry remains poorly documented in Europe. The EU Reference Laboratory for Honeybee Health sent a detailed questionnaire to each Member State, in addition to Kosovo and Norway. Based on data supplied by the National Reference Laboratory for honeybee diseases in each European country, a European database was created including the number and types of beekeepers, operation size and industry production. The total number of beekeepers in Europe was estimated at 620 000. European honey production was evaluated at around 220 000 tons in 2010. The price of honey varied from 1.5 to 40 €/kg depending on the country and on the distribution network. The estimated colony winter mortality varied from 7 to 28% depending on the country and the origin of the data. This survey documents the high heterogeneity of the apicultural industry within the European Union. The high proportion of non-professional beekeepers and the small mean

number of colonies per beekeeper were the only common characteristics at European level.

The tremendous variation in European apicultural industries has implication for any comprehensive epidemiological or economic analysis of the industry. This variability needs to be taken into account for such analysis and for future policy development. The industry would be served if beekeeping registration was uniformly implemented across member states. Better information on the package bee and queen production would help in understanding the ability of the industry to replace lost honey bee stocks.

Feeding sugar syrup and sugar candies with high levels of HMF (Hydroxy methyl furfural) is bad for honey bee vitality

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Feeding honey bees artificial carbohydrates is an important part of conventional and organic beekeeping management. These feeds can influence honey bee colony vitality. Enzymatic processing of artificial feed can result in different end products when compared to the breakdown products resulting from bees breaking down natural feeds. These products could have a negative effect on bee health. One breakdown product of particular concern is HMF. For this reason there is a need to standardise HMF (*hydroxymethylfurfural*) quantification methodologies. Using data obtained from previous feeding studies, we used the Winkler quantitative analysis to quantify HMF levels in products for feeding the bees before they were used. Our results found that levels of HMF in stored bee feed regularly exceeded the levels that are considered tolerable in honey. In one case that levels HMF were measured at c 222, 5 mg/kg. It is unclear what effect this level of HMF will have on bee health. Further studies quantifying negative effects are badly needed. If these levels do have a demonstrable negative effect we propose that concentrations of HMF should be required on the labelling of all bee feeds. Such labelling should be standardized and include recommendations for product storage and use.

Deformed wing virus and variation of polyprotein gene sequences in Lithuanian honey bees

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Pathogenic honey bee viruses have recently aroused particular interest as they are

also suspected to play an important role in colony decline. The deformed wing virus (DWV) is considered to be the most widespread bee virus in Europe. The data about distribution and presence of the honey bee virus in the Baltic countries, including Lithuania was absent. In this study, the presence of DWV was tested using the reverse transcription polymerase chain reaction. The study proves that DWV does not prevail in Lithuanian apiaries (in autumn of 2012 DWV was found in 17.29 % and in summer of 2013 in 5.47 % of colonies). However, it could affect the overwintering of honey bee colonies. Among the colonies recorded as infected with DWV in 2012, approximately 22 % did not survive over winter. Isolated polyprotein gene sequences of DWV were polymorphic and they were closely related with one of the sequences from the UK.

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First detection of Deformed wing virus of honeybees in Algeria

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Deformed wing virus (DWV) is a very common virus infecting honeybee in all developmental stages and castes and has become one of the most common honeybee viruses in the world. DWV causes characteristic wing deformities, shortened abdomens and a severely reduced lifespan of adult honeybees. Declines in honey bee (*Apis mellifera* intermissa) colonies in Algeria have been attributed by the beekeepers to the presence of DWV, when the examination of the colonies revealed the presence of adult bees with deformed wings. The aim of the present work was to investigate the presence of DWV in *Apis mellifera intermissa* from apiaries of different regions of northern Algeria using one-step reverse transcription-PCR (RT-PCR). Results show that, 40% of the apiaries examined were infected with DWV. An epidemiological survey on DWV and other honeybee viruses covering most part of the country needs to be done in order to show spatial dynamic in virus distribution and to determine the prevalence of honeybee viruses in Algeria apiaries and its role in the mortality of bees.

Viral infections in honey bee queens from Polish apiaries

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The goal of this study was to assess the virus status of 91 adult honey bee queens derived from Polish apiaries. Individual queens were examined for the presence of four honey bee viruses: deformed wing virus (DWV), acute bee paralysis virus (ABPV), chron-

ic bee paralysis virus (CBPV) and Israeli acute paralysis virus (IAPV). Individual queens were homogenized in liquid nitrogen, then total RNA extraction, one-step RT-PCR assay and agarose gel electrophoresis were conducted. The investigation showed that among 91 examined queens 76% were positive for at least one virus, 69% examined queens were positive for DWV, 57% for ABPV and 13% for CBPV. There were no positive samples for IAPV. Of the 91 queens examined, 19% were positive only for one virus (among them 71% were positive for DWV and 29% were positive for ABPV). Of all queens investigated, 57% had multiple viral infection of two or three viruses (50,5% and 6,6% of examined queens accordingly). In case of multiple infections, 51% of examined queens were positive for ABPV and DWV simultaneously, 12% for CBPV and DWV and 8% for ABPV and CBPV. This study demonstrated that a large percentage of adult honey bee queens from Polish apiaries harbor viruses, especially DWV and ABPV.

Comparative analysis of cuticular hydrocarbons of healthy and infected with deformed wing virus honeybee workers (*Apis mellifera* L.)

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In social insects chemical signals play an important role in the regulation of group homeostasis, but unfortunately this balance can be altered by parasite or virus infections. For example, in honeybees the ectoparasite *Varroa destructor* and endoparasite (*Nosema ceranae*) can modify a group of cuticular hydrocarbons involved in social recognition (McDonnell et al., 2013). We analysed the cuticular hydrocarbons (CHC) of honeybees collected from healthy and infected with deformed wing virus (DWV) colonies. The presence of DWV was confirmed by PCR analysis. Comparative analysis of CHC profiles by gas chromatography coupled with mass spectrophotometry (GC-MS) showed changes in healthy and DWV infected honeybees. We identified 31 of compounds detected on the honeybee cuticle in the different health condition groups. The average total CHC amount found on the cuticle of healthy honeybees was statistically greater than that of DWV infected bees. CHC profiles of healthy bees presented a relatively greater abundance of low molecular weight compounds, while the DWV infected bees had more higher molecular weight compounds. The principal components analysis of relative amounts of CHC grouped honeybees to the correct group and most of variance in the data set could be explained by the first function, which separated healthy bees from DWV infected bees.

Identification of viruses in *Varroa destructor* using proteomic methods

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The aim of the current study was to identify pathogenic viruses in *Varroa* sample

using proteomic methods. A p-Aminobenzamidine (p-ABA) ligand was used to separate proteins to affinity bound and unbound fraction. Three samples were obtained after the extraction and purification process: (i) supernatant of crude extract (supernatant before purification); (ii) unbound fraction (supernatant after purification); and (iii) proteins affinity-bound on the matrix of p-ABA column (purified proteins). Homogenized mite sample was examined using pI 3–10 two-dimensional gel electrophoresis (2DE; 10 and 14 %) and matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF)/TOF protein identification. The samples were finally analyzed using difference gel electrophoresis (DIGE). Applied purification process enabled identification of viral protein spots present in *Varroa*. The MALDI TOF/TOF protein identification enabled identification of protein spots corresponding to Kakugo virus, Deformed wing virus, *Varroa destructor* Macula-like virus and Acute bee paralysis virus. In addition, some results were similar to other viruses that could be identified by similarity. Moreover, some of the analyzed proteins were identified as honeybee hemolymph proteins. The event that viral proteins were identified in the *Varroa* protein sample proves that Kakugo virus, Deformed wing virus, *Varroa destructor* Macula-like virus and Acute bee paralysis virus are transmitted by *Varroa*. All of the identified proteins are useful as markers for future comparative studies and identification of honeybee diseases. Identification of honeybee hemolymph proteins within *Varroa* proteins point out on need of analysis of *Varroa* respecting the food source – honeybee hemolymph. This study was supported by Grant No. QJ1310085.

Different measures to prevent virus prevalence in reared bumblebees

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Israeli acute paralysis virus (IAPV) is lethal after injection in honeybees. It has also been reported in non-*Apis* hymenopteran pollinators such as bumblebee species, which can become infected when placed in the neighbourhood of infected honeybee hives. Furthermore, injection of IAPV particles resulted in bumblebee mortality within 7 days. Therefore we looked at different measures to prevent IAPV infection and bumblebee mortality. Eradication of IAPV is important, knowing that annually more than 1 million bumblebees colonies are sold for pollination purposes, and a virus-free status is desired for transport of bumblebees. We suggest three lines of action: 1) 15kGy gamma radiation of pollen to prevent the virus from entering the breeding facility, 2) proactive treatment with dsRNA targeting the IAPV genome to reduce viral infection prevalence, and 3) viral diagnostics to eradicate infected colonies. Progress on all three lines of action is presented.

The physiological pH of the bee's intestine after treatment with oxalic acid – impact on the development of the gut parasite *Nosema ceranae*

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Organic acids have become the treatment of choice to combat Varroosis in colonies of *Apis mellifera*. Oxalic acid for example is used by trickling a specific amount of 3.5% oxalic acid dehydrate in sucrose solution onto the honey bees between frames. It has been shown that the treatment of honey bees with oxalic acid dehydrate (dermal or oral) resulted in recovery of oxalic acid in the digestive system and haemolymph. The aim of this work is to understand whether organic acids affect the physiological pH of the bee's intestine (crop, ventriculus and rectum) in sublethal dosages. At first a standard pH value will be defined for the honey bee *Apis mellifera*. During the trial bees receive varying amounts of oxalic acid/sucrose solution in dermal or oral individual application. In defined intervals the treated bees are frozen and dissected. The pH of the liquid contents from parts of the digestive system is then measured with a pH surface electrode. This electrode enables the pH measurement of very small amounts of liquid. The results will be compared to the standardized pH as well as the respective control groups. Germination of *Nosema apis* is susceptible to different acidity levels. Secondly, it will be discussed whether a possible change in the ventriculus pH value influences the infestation rate of *Nosema ceranae* in artificially infected honey bees.

***Crithidia mellificae*, cultivation of field isolates and influence on the host immune system.**

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The honey bee *Apis mellifera* is challenged by severe colony decline, this includes losses caused by CCD and winter mortality. Pathogens play an important role in these abnormal losses. The trypanosome *Crithidia mellificae* has, together with *Nosema ceranae*, recently been identified as a predictive marker for winter mortality. In our study, we took a closer look at *C. mellificae* and the relation with its host. Field isolates of *C. mellificae* were cultivated from honey bees and solitary bees. The cultivated isolates all belong to the haplotype A and they all express *gp63*. In *C. fasciculata* this protein is needed for the survival in the gut of the insect host. A similar function for the homologue in *C. mellificae* is supposed. In a second part of the study we examined the honey bee immune response to *C. mellificae*. With Q-PCR and an *in-house* developed microarray the differential gene expression of a *C. mellificae* positive colony to a negative colony was measured. A significant upregulation of *Hopscotch*, *Imd* and *IRS* and a significant downregulation of *apidaecin* and *prophenoloxidase* was noticed. We conclude that the JAK/STAT and Imd pathway

play a role in the immunity against this organism. In addition the lytic antimicrobial peptides are upregulated, although not significantly. *IRS* is known to play a role in the preference of foraging bees to nectar or pollen. We suggest that *C. mellificae* possibly influences the feeding behavior of the honey bee, resulting in an upregulation of this gene.

Evaluation of different substances for control of *Nosema ceranae* under field conditions

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In the present study various pharmaceutical and natural products were applied to control a natural infection of honey bee colonies by *Nosema ceranae*. These compounds were selected for their known bactericidal and fungicidal properties.

Two groups of *Apis mellifera iberiensis* colonies, naturally infected with *Nosema ceranae*, were randomly located in two different apiaries 500 meters apart from each other with the same floral conditions and climatology. One received the products to evaluate and the other served as untreated control. Honey bee colonies of the treated group received syrup and candy supplemented with different products: tannic acid, resveratrol, toltrazuril and fumagilin. Honey bee colonies of the untreated control group received syrup and candy or nothing. Different type of samples (house and forager honey bees) along different seasons were collected and PCR analyzed (for detecting *Nosema* spp. Tripanosomatids, Neogregarines, and the main bee viruses) to determine the efficacy of the substances tested and the evolution of *Nosema* infection and honey bee colonies strength.

The percentage of parasitized honey bees by *N. ceranae* in the colonies treated with any of the substances tested was significantly lower than the percentage of parasitized bees in control colonies. The role of other pathogens detected (Tripanosomatids, BQCV or DWV) in the evolution of the colonies has been also discussed.

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Low levels of recombination in *Nosema ceranae* proved by single genome amplification (SGA)

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Nosema ceranae is a microsporidian parasite that infects the ventricular cells of honeybees. Recent studies performed with PCR amplification and subsequent cloning enabled the detection of high levels of haplotype diversity within honeybee colonies and of signs of recombination at some of the studied loci. However, given that the PCR amplification of mixed samples may generate chimeric haplotypes, the evidence for genetic exchange should be interpreted with caution. In this work we applied a terminal dilution technique based on the amplification of single DNA molecules (SGA), that avoids the generation of PCR-induced artefacts, to assess the occurrence of recombination at the *PTP2* locus of *N. ceranae* in five isolates, previously studied by PCR/cloning.

The sequences thus obtained confirmed the existence of high levels of variation of the parasites within honeybee colonies and exhibited low but significant rates of recombination; four-allele combinations were found at coincidental intervals with those observed in clones, which argues against the possibility of these being artefacts of PCR amplification from mixed templates and corroborates the existence of DNA exchange in *N. ceranae*. This finding opens new questions about the biology and life cycle of this putatively asexual parasite, as this process confers a wider diversity and plasticity that may derive in a better capacity to adapt to different hosts and environments, promoting a faster expansion of this honeybee parasite.

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Patterns of genetic variation at the *PTP2* and *PTP3* loci in a worldwide sample of *Nosema ceranae* isolates

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Nosema ceranae is a microsporidian parasite that causes nosemosis type C in *Apis mellifera* and other hymenoptera. Epidemiological data suggests that *N. ceranae* jumped from its original host, *Apis cerana*, to *A. mellifera* and spread rapidly worldwide, although no population genetic analyses have confirmed this hypothesis so far. In order to obtain more information about the recent evolutionary history of this parasite, we investigated the

patterns of genetic variation of *N. ceranae* at two single copy loci (the polar tube proteins *PTP2* and *PTP3*) in DNA isolates of different hosts from all over the world. The results revealed evidence of recombination among common haplotypes and similar levels of genetic polymorphism (pS ~ 1%) across isolates. Although most of the variation found was due to differences within isolates (= bee colonies), there was significant differentiation between *N. ceranae* collected from Asian honeybees (*A. cerana* and *Apis florea*) and those obtained from *A. mellifera*. This, together with the significant excess of low frequency variants detected among Western honeybee parasites, but not from those of Asian honeybees, supports the hypothesis of a recent and rapid spread of *N. ceranae* throughout *A. mellifera* colonies, in which the virulence of this pathogen is causing dramatic population declines.

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***Crithidia mellifica*: Distribution, prevalence and intensity in Switzerland.**

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The gut protozoan *Crithidia mellifica* is an associate of honey bees (*Apis mellifera*) and has been implicated in host colony winter losses (Ravoet *et al.* 2013). However, very little is known about its actual impact on honey bees and its local fine-scale distribution, prevalence, and intensity. Here we conducted a *C. mellifica* survey in Switzerland. In 2011 and 2012, adult honey bee workers were collected in spring (April), summer (June), and autumn (October) from 18 beekeeping operations throughout Switzerland. During each sampling and at each operation, 60 workers were collected from four colonies each. The samples were pooled on colony level and evaluated using routine qPCR. The data show that *C. mellifica* is highly prevalent with 87.6% infected colonies in the year 2011 and 82.5% infected colonies in the year 2012. Furthermore, the parasite was widely distributed throughout the country, with at least one colony in each beekeeping operation infected. In both years, the intensity of *C. mellifica* ranged from a relative starting concentration per sample N0 of 5.4×10^{-13} to 1.4×10^{-6} and was significantly lower in spring compared to summer and autumn ($p < 0.001$). The data give clear evidence that *C. mellifica* is highly prevalent and distributed all over Switzerland. Furthermore, the data suggest seasonality of the parasite because intensity was significantly lower in spring compared to summer and autumn. In conclusion, the distribution and prevalence of *C. mellifica* has been underestimated so far, thereby creating demand for more studies on this ubiquitous parasite of honey bees.

Differential immune response of the honey bee *Apis mellifera iberiensis* after experimental infection with *Nosema ceranae* from Spain and Uruguay

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Nosema ceranae is a novel pathogen of the honey bee *Apis mellifera* and it has been associated with colony losses in Spain. However, the role of the microsporidia in this phenomenon is controversial. The objective of this study was to compare the immune response of the Spanish honey bee *A. mellifera iberiensis* after experimental infection with *N. ceranae* spores from Spain and Uruguay, a country where *N. ceranae* is widely distributed but no massive colony losses have been reported. *N. ceranae* variants from both countries were characterized by ISSR, showing different genetic patterns. A group of ten bees was individually infected with *N. ceranae* spores from Spain, other group with *N. ceranae* spores from Uruguay and a last group was kept as a control. Seven days post-infection bees were sacrificed, RNA was individually extracted, retrotranscribed and expression of vitellogenin and different genes related to the immune response were analyzed by real time PCR. Expression levels of β -actina and RPS5 were used for relative quantification. Bees infected with both *N. ceranae* variants showed reduced expression of genes coding for abaecin and phenoloxidase compared to control bees. Evenmore, bees infected with Uruguayan variant also showed reduced expression levels of deterin and imd compared to control bees. Finally, no differences in the expression of defensin, glucose deshidrogenase, hymenoptaecin and vitellogenin were observed between groups.

These results confirmed that *N. ceranae* causes immune-suppression and that different genetic variants of the pathogen can induce differential responses, which could be related with differences at colony level.

A new qPCR primer set for *Nosema ceranae* based on the highly conserved protein coding gene HSP70

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Nosema ceranae is an asian microsporidian parasite, recently spread across the world and responsible for the disease called Nosemosis type C, often linked with colony collapses. The identification of this fungus cannot be done by microscopy hence the importance of using a reliable molecular method. Several PCR based methods have been developed so far, all based on sequences of the 16S rRNA gene. However, recent studies point out that this gene is present in multiple copies in the genome and it is extremely variable, making

it a poor candidate for PCR assay design. Here we present a qPCR assay based on the single copy, highly conserved gene HSP70, coding for an heat shock protein of the parasite. Multiple clones of the gene were sequenced and aligned to choose a low variability zone for the primers and TaqMan® probe design; an in-silico evaluation of specificity was made using nucleotide BLAST ® algorithm. A short fragment size (69 bp) was selected for the amplification, meeting the requirements for a Real-Time PCR reaction. In order to achieve an absolute quantitation the use of standards of known concentration is needed. In this assay we used as standards serial dilution of a synthesized oligonucleotide (IDT Ultramer®) , having the sequence of the amplicon. The reaction showed proper efficiency and a sensitivity of approximately 2 copies. Therefore, we propose this assay as a solid and reliable molecular tool for *Nosema ceranae* detection and quantification. The study has been conducted within the BEENET project.

***Nosema ceranae* gene expression to study the intracellular cell cycle in the honey bee host**

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Nosema ceranae is a Microsporidian causing important losses in honey bee colonies in Spain. It is known that honey bee infection takes place after ingestion of mature spores, and its life cycle is completed in less than 3 days. However there are some aspects of its biology that still remain unknown, mainly during the first stage of infection. In this study we study some aspects of the intracellular cycle in these initial moments by studying the expression of some genes of *N. ceranae*.

Bees were infected with 100,000 spores per bee and their ventriculi were collected after 0, 8, 12, 24, 36, 48 and 72 hours post infection (p.i). We selected the genes encoding for the polar tubule protein 3 (*PTP3*), the *RNA polymerase* (RNAP) and the *Heat shock protein 70* (Hsp70) as targets and we studied the level of expression in the infected bees. The expression was analyzed by quantitative PCR by determining the Cp value.

The *PTP3* gene expression was the earlier detected. It was first detected after 36 hours of infection, indicating that *PTP 3* is forming during the development of the *N. ceranae* sporoblasts. The expression of Hsp70 that was expected to be detected earlier since it encodes for a structural protein was however detected later (48 h post-infection) and the same happened for the RNAP gene. These results agree with the previous data, confirming that the cell cycle is completed in less than three days.

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Fumagillin treatment of honeybee's colonies during winter doesn't affects neither *Nosema ceranae* infestation level nor colony size in the spring

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Colony losses or poor development in the spring in honey bees have been claimed by Uruguayan beekeepers that moved their hives to *Eucalyptus grandis* plantations during the fall. *Nosema ceranae* is suggested as an important factor causing those problems. The efficacy of four fumagillin treatments on *N. ceranae* control and their impact in colony size were evaluated. Ten colonies for each treatment were brought from *E. grandis* plantations. Treatments were administered between June the 1st and June 19 as follows: 1) 4 applications of 30 mg fumagillin in 250 mL of 1:1 sugar syrup, 2) 1 application of 120 mg fumagillin in 5000 mL of 2:1 sugar syrup, 3) 4 applications of 30 mg fumagillin in 100 mL of 1:1 sugar syrup sprayed over the bees and 4) 4 applications of 90 mg fumagillin in 250 mL of 1:1 sugar syrup. Three controls were done administering to the colonies the same amount and concentration of sugar syrup used in the treatments, without the fumagillin. The nose-mosis level 10 days after of receiving fumagillin has diminished ($P = 0,006$). In September 25, no differences between colonies that received fumagillin and control colonies were found in respect to level of nose-mosis ($P = 0,21$), bee's population ($P = 0,18$) or brood area ($P = 0,60$). Thus, in the conditions of this study, fumagillin have controlled temporarily *N. ceranae* but didn't signify a benefit neither in the survivor nor in the size of the colonies.

Food additives as alternative treatments for *Nosema ceranae* disease

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Honeybee colonies affected by eurythermal microsporidium *Nosema ceranae* is considered as an emergent disease that is posing a major threat to the health. Due to its detrimental effects on both strength and productivity of honeybee colonies, an appropriate control of this disease is advisable. Prolonged fumagillin treatment may contribute to developing resistance and declining concentrations of this preparation may exacerbate disease rather than suppress it. Furthermore, the use of antibiotic is not allowed in the EU member states. For that reason, the need for the utilization of food additives arises as alternatives to control this disease. In the present study three food additives with herbal, protein and mineral basis (NozevitPlus, BeeWell Aminoplus and Eko ZeoPet), as potential therapeutic agents, have been tested to control nose-mosis type C in honey bee colonies. Also, the histopathological structure as well as content and distribution of mucosubstances and histochemical activity of aminopeptidase and non-specific esterase, both important enzymes of intermediate metabolism in the mid gut of honeybees, were investigated. The

number of *Nosema* sp. spores was determined by counting spores using a Bürker-Türk haemocytometer, and to identify the species of *Nosema* sp. molecular diagnostic methods were used. For the purpose of describing neutral, acid and sulphate mucopolysaccharides we used the Periodic Acid-Schiff Reaction, Alcian blue-specific and Toluidine blue-specific staining. Degreased cuts of midgut were stained according to the HE method in order to determine the general morphological characteristics of the tissue, and stained with special stains for determination of enzymes activity. Results of different treatments were mutually compared and possible effects of these treatments on the honeybee digestion are discussed.

The microsporidian *Nosema ceranae* facilitates Black queen cell virus infections in honey bees (*Apis mellifera*)

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Host-parasite interactions are a major evolutionary force and have received considerable attention. However, hosts are often infected by multiple parasites and numerous specific parasite-parasite interactions are not yet fully understood. Here we investigate the potential effect of the nearly ubiquitous intracellular microsporidian parasite, *Nosema ceranae*, on natural black queen cell virus (BQCV) infections in the honey bee (*Apis mellifera*). We found that *N. ceranae* inoculated workers displayed higher BQCV titers, thereby suggesting that the presence of the microsporidian can activate latent virus infections. This is in line with earlier reports of the association of *Nosema apis* and BQCV. However, in individual bees *N. ceranae* spore counts and viral loads were not correlated, indicating that the microsporidian potentially promotes BQCV indirectly via general effects, for example via immunosuppression.

Fumagillin control on *Nosema ceranae* (Microsporidia: Nosematidae) infection in Honey Bee (Hymenoptera: Apidae) Colonies from Argentina.

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The objective was to evaluate the effectiveness of fumagillin on *Nosema ceranae* control in Argentinian east-central honey bee colonies. Honey bee hives were assigned to 3 different fumagillin control strategy groups. A preventive strategy with hives monthly

treated, a monitoring strategy with hives treated according to *N. ceranae* level threshold and control group with untreated hives. Apiaries were monitored monthly during fall-winter 2009 and 2010 and *N. ceranae* spore level and honey bee colony strength measures were estimated. Repeated-measures ANOVA and Duncan test were used to compare spore level and hive strength variables between control and fumagillin treated groups. Fumagillin treated colonies reduced *N. ceranae* spores load during 2010 compared to untreated colonies. However, there were no significant differences between treated and untreated groups for colonies strength measured as bee adult population, bee brood, honey or pollen availability. Fumagillin treatment reduced *N. ceranae* spore level, nevertheless seems to have little effect on colonies. The bee population during winter was reduced in treated as well as in untreated colonies. Our results contribute to clarify that fumagillin treatment should be at least reviewed and that further research should be conducted in order to get a more complete perspective of Nosemosis disease.

What Looks Like Nosema Spore Is Not Necessary A Nosema!

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In recent years there is growing concern worldwide about the health of the honeybee, specifically in relation to the phenomenon of Colony Collapse Disorder (CCD). Nosemosis is one of the most widespread honey bee diseases caused by Microsporidia *Nosema apis* and recently by *N. ceranae*. *N. ceranae*, originally parasite of *Apis cerana* recently spread to many regions, substituting the former pathogen, *N. apis* in *Apis mellifera* and also attacking bumble bees. At high infection levels *Nosemosis* correlate with colony decline. Although its global significance of nosemosis yet to be determined. In Spain *N. ceranae* was indicated as the main cause of the CCD (Higes et al., 2008; 2009), raising questions regarding significance of preventive and therapeutic treatments. Decision policy for any kind of treatment is usually based on routine pathogen monitoring. The infection level of *Nosema ceranae* and *N. apis* can be precisely identified by molecular techniques, using specific primers. However, this method is not commonly accessible and the infection is generally identified by beekeepers by counting the number of easily identified *Nosema* spores in the gut smear content under a light microscope.

We will present data that raise concern over the validity of identification *Nosema* infection based on Microscopic analysis alone at least in some geographical areas or seasons. Microscopic analysis of the bee gut content of honey bees supplementary fed by commercial irradiated pollen revealed some forms that under the light microscope were similar to *Nosema* spores. Same structures were also found in pollen samples used as food. However, results by PCR analysis of the same pollen samples with specific primers for *N. apis* and *N. ceranae* were in fact negative. An Electron microscopic analysis revealed the suspicious objects as amorphous structures, which were later confirmed as starch drops by Lugol's Iodine staining, a secretion released from pollen grains of genus *Cistus*. The members of this genus of plants are very common in the Mediterranean region indicating

that the danger of miss interpretation of Nosema analysis in honey bees feed with these plants is high. Whether the same phenomenon occurs in pollen of other plant groups, remains further studied.

First Detection of fluvalinate resistant mites in Algeria

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Varroa destructor Anderson & Trueman, is considered as a major problem for the beekeeping sector, not only for *Apis mellifera* L. in Algeria but also worldwide. In cases of no control it can cause severe problems that may end in the death of honeybee colonies. Fluvalinate is the predominant compound used in Algeria to control *V. destructor*, its constant application has caused the appearance of resistant mite populations to this product in several parts of the world. This study was conducted to detect the possible existence of populations of resistant mites to fluvalinate in the area north-center of Algeria. To determine the mites mortality percentage to the fluvalinate, they were exposed to strips of 2.5 x 1.0 cm. Varroa mortality in apiaries treated with fluvalinate was 41.23%, lower than the 81.51% mortality obtained in apiaries that only received an alternative treatment. A significant difference ($P > 0.05$) was found between two mortality of Varroa. These results show for the first time the existence of *Varroa destructor* populations resistant to fluvalinate in Algeria. These results are essential for beekeeping sector not only in Algeria but also in Africa since very little data is available on this issue in the northern parts of Africa.

A new formulation with oxalic acid for *Varroa destructor* control to be applied during permanent brood presence on *Apis mellifera* colonies

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An organic product based on oxalic acid was evaluated for use in Varroa control under spring/summer climatic conditions in Argentina. The formulation consist in four strips composed by cellulose (45 cm of large x 3 cm of width x 1.5 mm of thickness) impregnated with a solution based on oxalid acid formulated by Cooperativa de Trabajo Apícola Pampero Ltda. 48 beehives were used to asses the product efficacy. The residuality of the product in honey, bees and wax also was tested. Colonies used were previously standarized for bee population, brood area and honey and pollen stores. Hive bottoms specially adapted

for collection of dead mites were placed in each colony. In each trial the respective control groups without oxalic treatment were performed. At the beginning of the experiment four strips of the new formulation were applied to the colonies belonging to the treated group. Each strip was placed between frames 2, 4, 6 and 8 of the brood chamber. Falling mites were counted after 7, 14, 21, 28, 35 and 42 days. After the last count, the strips were removed and colonies received two flumethrin strips (registered trade name: Flumevar®, supplied by APILAB SA, Argentina). This shock treatment had a 40 five days of permanence inside colonies. Falling mites also were counted throughout this period. Bee population was registered during the field trials. Average final efficacy of the organic product was 95.9% with a low variability. No queen, brood, or adult honeybee mortality was registered.

The first evaluation of Flumvar efficacy in control of Varroasis in honey bee colonies at Iran

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Varroasis is one of the most important disease of honey bee which is caused by the *Varroa jacobsoni*. This mite destroy colonies by feeding via pupa nests. *Varroa* infest the larva in nests and infested larva produce weak adult honey bees. In Iran several chemical compounds are used for control of varroasis.

In this study, the efficacy of FLUMVAR (flumethrin) was evaluated in compare with other flumethrin product (BAYVARROL). Three experimental groups including group C (control) group F (Flumvar) and group B (Bayvarrol) which each group including 45 bee hives, were evaluated.

Before using the Flumvar and Bayvarrol, primary infestation to *Varroa* were determined by detecting and counting of mites on 100 adult honey bees in each hive of groups in September of 2013. Primary infestation of bee larva were determined also by consideration of 50 larva nests in each hives. Then Flumvar and Bayvarrol strips were used as their prescription and 40 days after drug administration, Varroal infestation rate was determined again. Results was showing the significant decreasing of varroal infestation rate in both treatment groups. ($P < 0.01$) The efficacy of Flumvar and Bayvarrol were evaluated and there was not significant differences. ($P > 0.05$)

Flumvar and Bayvarrol were reduce primary infestation in adult bees from 11.6 to 0.73% and 11.8 to 0.87%, and their efficacy were 93.71% and 92.63% respectively. So the efficacy of Flumvar in control and treatment of varroasis was perfectly acceptable.

Essentials oils from Portuguese aromatic flora as a potential alternative for *Varroa destructor* control

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The hemophagous mite *Varroa destructor* Anderson & Trueman is a honey bee colonies parasite, nowadays considered the greatest threat for apiculture worldwide. It was recently regarded, with the viruses they vector, as a possible cause of Colony Collapse Disorder. Besides ineffectiveness, the regular use of chemical treatments accelerated mite population resistance or tolerance development, added by high costs and labour, and residues accumulation in apiculture products. Aromatic plants products, mainly their essential oils (EOs) and components, offer an attractive alternative to synthetic acaricides for *Varroa* control.

EOs isolated from Apiaceae, Asteraceae, Lamiaceae, Myrtaceae and Poaceae species from the Portuguese flora were assessed for acaricidal activity. The EOs were isolated by hydrodistillation and analyzed by GC e GC-MS. The EOs effect on varroa and on the honey bees was evaluated by the complete exposure method, using both varroa-parasitized newly emergent bees and adult bees. Different EOs concentrations were tested, with five replicates *per* experiment (n = 25 bees/tested concentration). Blank, negative and positive controls were included. *In vivo* bioassays were done under controlled temperature and RH, and mites mortality and the toxicity effects on bees assessed after 6, 12, 24 and 48h.

Some EOs showed repellent and/or lethal effect on mites. Running experiments evaluate the EOs concentrations with less toxic effects on the bees and the minimum lethal dose for *Varroa*.

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Varroa infestation and presence of viruses in honeybees colonies without varroosis treatment, in temperate and subtropical climates in Argentina

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In the framework of the network for evaluation of honeybee stocks for Varroa tolerance in Argentina Varroa levels and the presence of virus were analyzed in local stock (LS), comercial(CS) and selected colonies for varroosis tolerance (SS) in temperate and

subtropical climates. In subtropical conditions LS of different haplotypes (C1 and A1) registered similar values of parasitism in adults bees and brood (3 % and 1,3% for haplotype C1 and 2,8 and 1,8 % in A) with a survivor of 50% and 70% respectively. Deformed wing virus (DWV) was detected in colonies with Varroa prevalences in adults bees of 10,5% and more than one virus (DWV and CBPV) with average levels of phoretic varroa of 19%. In temperate climates phoretic varroa after six months of evaluations was 5.8% in colonies LS and 9% in colonies CS with a colony survivor of 58% and 65% respectively. Colonies SS presented values of 2.3% in the same period with a colony survivor of 84 %. All colonies were C1 haplotype. DWV was detected with average levels of phoretic varroa of 12,6% and both viruses (DWV and CBPV) with average levels of phoretic varroa of 5,3%. No virus was registered in SS colonies. European honeybees in both climates presented similar values for varroosis parasitism. DWV prevalence was similar in both climates but other viruses were detected in temperate climate with lower varroa infestation levels. The ABPV was detected only in colonies located in temperate climate.

Identification of haplotypes of *Varroa destructor* in Uruguay by a new technique of RFLP

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Varroa destructor is an ectoparasite of honeybee that causes big losses to beekeeping industry. At least two possible geographic origin have been described that can be distinguished by their mitochondrial haplotype: Korean (K-haplotype, cosmopolite distribution), and Japanese (J-haplotype, more restricted distribution). It has been suggested that the K-haplotype is the most virulent. In Uruguay most colonies die if they are not treated with acaricides, but in the East of the country many colonies survive without treatment. The cause of this difference is unknown and could be due to the infestation by *V. destructor* from different haplotypes. Although J-haplotype arrived to Southamerica in the 70s, the K-haplotype is the only variant detected except for J-haplotype found in particular sites of the Brazil. Considering the proximal geographical contact with Brazil, J-haplotype could be present in Uruguay. We identified haplotypes of mites taken from 7 regions of Uruguay, including the region where *V. destructor* does not cause severe damage to the colonies, by a new system of RFLP technique. We designed a new pair of oligonucleotides to partially amplify the mitochondrial COXI gene, and digested the PCR-products with the restriction enzyme EcoNI, which differentially cuts each haplotype (twice J-haplotypes, thrice K-haplotypes). This system, unlike other previously described, does not depend on the J-haplotype as control of the digestion. We found that all *V. destructor* analysed correspond to K-haplotype. These results show that the difference in *V. destructor* virulence in Uruguay is not explained by mite haplotype.

Proteomic analysis of the effect of parasitization by *Varroa destructor* on the honeybee, *Apis mellifera*

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The Western honeybee is an insect of extreme economic importance responsible for around 80% of global pollination annually. Bees live in densely populated colonies of up to 50,000 individuals and these conditions provide an ideal environment for the proliferation of pathogens and parasites. One of these, the hemophagous mite *Varroa destructor*, has been shown to cause devastating effects at the individual and colony level. *V. destructor* has the ability to parasitize both the adult and larval stages of the bee, with its life cycle is completely dependent upon that of the developing larvae.

A novel proteomic analysis was applied to examine the effect of parasitization on the developing larvae. The results indicate that a large proportion of proteins were changed in expression following parasitization by *V. destructor*. A number of proteins involved in normal cell metabolism were shown to be decreased in parasitized larvae, which could impair the emergence of a viable worker. Certain proteins involved in detoxification and the stress response such as Glutathione S-transferase and Heat shock proteins were found to be unregulated in response to parasitization by *V. destructor*, indicating the weakening effect of the mites on their development.

These results indicate that the presence of *Varroa* in the cell during development to adult could lead to the emergence of less viable workers with a possible knock on effect on the colony as a whole.

Resistance of *Varroa destructor* to Apistan and Bayvarol in *Apis mellifera intermissa* colonies.

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Efficacy of Fluvalinate and Flumethrin molecules against *Varroa destructor* was established on bee colonies in Central Algeria through field tests using Apistan®, Bayvarol® and, as a control treatment, Apiguard®. The average efficacy was found, respectively, to be 53% and 41%, far from real therapeutic value, probably due to populations of *Varroa* mites resistant to Pyrethroid treatment.

Biochemical alteration in cuticle and haemolymph of honeybee (*Apis mellifera intermissa*) parasitized by *Varroa destructor*

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In order to study the interaction between the host (honeybee) and the parasite (*Varroa*), the aim of the work was to study the influence of *Varroa destructor* on the biochemical composition of the cuticle and haemolymph of bees pre-emerging (AE) *Apis mellifera intermissa*. These categories were classified according to the intensity of parasitization: bees with 1-3 mites, 4-6 mites and bees without mites (control). These experiments were taken in the apiary of Isser (36° 43' N., 3 ° 40' E.) (Boumerdes) in the summer. For the estimation of the cuticular abdominal protein-chitin content in the healthy and parasitized samples by *Varroa*, the method of Bordoreau & Anderson (1978) was employed. The hemolymphatic concentration of the protein and carbohydrate were determined using the method of Bradford (1976) and Duchateau & Florkin respectively.

The results showed that the mite infestation had a significant impact on the cuticle components. The cuticular abdominal protein content of slightly infested pre-emerging stage (1-3 *Varroa*) was reduced by 21, 73%. This content decreased by about 42, 02% in the heavily infested bees (4-6 *Varroa*). The chitin content of the heavily infested pre-emerging stage in comparison to control pre-emerging was reduced by 24%. However, a slight parasitism has no significant effect on the chitin content. A significant decrease in protein emerging worker bee was noted. The haemolymph carbohydrates content of the slightly and heavily infested pre-emerging stage in comparison to control pre-emerging was reduced by 33, 8% and 49, 2% respectively.

Reproduction of *Varroa destructor* affected by virus infections?

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The ectoparasitic mite, *Varroa destructor*, is the major pest of the European honey bee, *Apis mellifera*, especially due to its role as a vector for several honey bee viruses. In the citrus red mite (*Panonychus citri*) and in the European red mite (*Panonychus ulmi*), viruses can play an important role in the regulation of mite populations in citrus and peach orchards respectively. However, it is not known whether viral infections may also have an impact on the fertility of *V. destructor*. Here we investigate whether variation in *V. destructor* fertility may be a consequence of ongoing viral infections. We took advantage of natural infections with the ubiquitous deformed wing virus (DWV), that is vectored and replicates in the mite, and expected that high virus titers may compromise mite fertility. Worker brood samples were taken near Oslo, Norway in autumn 2013 (9 colonies at 2 apiaries) and in spring 2014 (24 colonies at 5 apiaries). Then, brood cells were dissected

to identify reproductive (N=18 in autumn, N=22 in spring) and non-reproductive mites (N=12 in autumn, N=56 in spring). All mites and their corresponding pupae were analyzed with routine qPCR for DWV and other viruses known to be associated with *V. destructor*: ABPV, IAPV, KBV, SBV, LSV-1, LSV-2, and VDV-1. Only DWV was found, but none of the other viruses. The first results suggest no significant differences in DWV loads between reproductive and non-reproductive mites or their corresponding pupae. So far, the data therefore suggest that *V. destructor* fertility may not be compromised by ongoing DWV infections.

Importance of a closed bottom board during *Varroa destructor* treatment using volatile substances.

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Control of the *Varroa destructor* mite is necessary to guarantee the health of honey bee colonies in Europe. Due to increased resistance problems with several miticides, a high interest exists for adopting alternative control strategies using volatile substances like formic acid or thymol. Professional beekeepers typically use hive boxes with an open bottom board made of metal mesh in order to provide good ventilation during transport; normally no possibility exists to close this bottom board. Here using 58 colonies we examined the influence of bottom board type (i.e. open versus closed) on the efficacy of formic acid treatments performed during two consecutive summers. We found that treatment efficacy was higher for colonies maintained in hives with a closed bottom board versus an open one in both years (+15% and +8%, respectively). We therefore can conclude that it is important to close hive bottom boards when treating honey bee colonies for *V. destructor* using volatile substances.

Development of cage tests with *Varroa destructor* parasitized honeybees for evaluating the effect and the compatibility of several acaricides.

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Managed honeybee colonies in temperate climates usually collapse within 1-3 years if a consistent *Varroa* treatment is lacking. However, some varroacides can cause resistances if they are used consecutively and alternatives are urgently required.

For evaluating potential solutions concerning their effects on *Varroa destructor* and their compatibility towards honeybees we developed a method of cage tests. For that purpose single mites were carefully transferred to the abdomen of healthy bees. Ten bees with

their attached *Varroa* mites were kept in one cage with sugar solution (ad libitum). Series of cages were put into an incubator with controlled temperatures and air moisture. Over a certain period of time (72 hours) these cage tests should guarantee a very low bee mortality rate as well as a high survival rate of *Varroa* mites. In comparison to such control groups without application of solutions we tested admitted varroacides as toxic standard for *Varroa* mites. With our developed cage tests we showed a possible test for evaluating several acaricides as potential varroacides and we could confirm that admitted varroacides can be considered as possible control methods.

Evaluation of Hygienic Behavior in Honeybee Hybrids *Apis mellifera* L. in Egypt

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To study the hygienic behavior of different lines of honeybee, 12 honeybee colonies headed by queens from with different lines and hybrids of honeybees (Car. x Lam., Lam. x Car., Lamarkii and local carniolan hybrid). Worker bees of all experimental groups except (Car. X lam.) began removing dead brood during the first two hours. The number of the removed dead brood and the hygienic behavior increases after four hours where the HB. Was highest for Lam. x Car. followed by Lamarkii, local carniolan hybrid and Car. x Lam. (13.8, 8.1, 7.71 and 2.39% respectively). After 24 hours of introducing dead brood all tested experimental groups removed more than 80% of dead brood. Local Carniolan colonies seem to be the lowest responded colonies to remove the dead brood (82%) in comparison with the tested hybrids which raised the efficiency of the colonies to remove the dead brood areas.

Quantification of peptide apidaecin in honey bee bodies or tissues

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Honey bees respond to microbial pathogens by a complex network of immune signalling and responses. A particular part of bee immunity is comprised by its humoral part, which consists of enzymes, lectins and antimicrobial peptides (AMP). Several AMP, namely apidaecins, abaecin, defensins and hymenoptaecin, were detected in the bee haemolymph. Their expression is triggered by microbial challenge through Toll, IMD-JNK or JAK/STAT signalling. Studies on bee AMP concerns mostly with changes in AMP gene expression, but only a limited number of reports on the quantification of AMP peptide levels have been published, generally using high numbers of bees immunized with

bacterial pathogens.

Apidaecins are heat-stable non-helical proline-rich cationic peptides, whose quantification is challenging due to their high basicity. We have built up a purification protocol for apidaecins 1a and 1b combining two SPE steps performed in a micro-column setup. Peptides were enriched using a cation-exchange sorbent and the eluate was desalted by on C8-sorbent. Quantitative analysis was done by a nanocapillary liquid chromatography connected to an ultrahigh resolution mass spectrometry (nanoLC-UHR-MS). The method allows to quantify apidaecin 1a and 1b in whole single bee bodies, haemolymph or individual parts of bee bodies. Apidaecin levels varied with bee age from 0 to 518 ng per bee, while the haemolymph concentration ranged from 32.4 to 68 ng/μl. The application of optimized protocol of apidaecins quantification will contribute to future studies on AMP role in bee immunity.

Occurrence of abnormalities and diseases in newly mated and old honey bee queens

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Observation of nonacceptance, frequent replacements and reduced performance of honey bee queens (*Apis mellifera ligustica*), induced to analyse newly mated and old bee queens to detect the causes. The study was carried out on 99 newly mated bee queens, from queen breeders, and on 109 older bee queens or at the end of their career, which showed a poor efficiency and were obtained from beekeepers. The bee queens were dissected to check their reproductive system status and/or the presence of various problems. Anomalies and/or diseases were observed in 39.39% of newly mated bee queens and in 75.23% of older ones. Absence, hypoplasia or atrophy and melanosis of ovaries; absence of oviducts; egg anomalies; missing, abnormally shaped, double or discoloured spermathecae; absence of or abnormal sperms; and enteroliths; also in association with a change in tissues were common. *Nosema* spp. spores were detected in five bee queens and in most of the accompanying workers when present. Ciliate protozoa were present in the spermatheca of 22 newly mated and 2 older bee queens. The observed differences permitted to highlight the causes of the reduced performance of the bee queens and of the hives they came from. The information obtained would prove useful to improve queen breeding techniques.

Sublethal effects of oxalic acid on *Apis mellifera*: motoric activity

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This experiment represents a part of the investigations concerning sublethal effects of oxalic acid on the vitality, behaviour and longevity of *Apis mellifera*. At this stage the effect of topically applied 3.5% oxalic acid dehydrate in sugar water 50% (OAS) or glycerol 45% (OAG) on motoric activity was examined.

Effects were evaluated 5, 24, 48 and 72h after topical application of 5µl of OAS and OAG, respectively (n=40). Controls received sugar water 50% (S) and glycerol 45% (G), respectively. Honeybees were tested in an open-field-like apparatus (30 x 30 x 5 cm) standing vertically and illuminated from above. The back area was divided into squares of 5 x 5 cm. After introduction to the box honeybees were allowed to move for a 3-min observation period. The bee's position and its behaviour was recorded every 3 s.

For data analysis, a One Way ANOVA on Ranks was used ($p \leq 0.05$).

Compared to an untreated reference group, significant differences could be found: bees treated with OAS or OAG showed increased self-grooming up to 24h after treatment ($p \leq 0.009$, $H \geq 13.398$, $DF=4$) and bees treated with G showed significantly less walking 5h after treatment ($p=0.003$, $H=15.674$, $DF=4$). However, amongst the treatment groups no significant differences in the behaviour could be found.

So far sublethal effects of oxalic acid on brood care, activity, food ingestion and longevity could be found. However, in this experiment the treatment had, with the exception of increased self-grooming, no effect on behaviour and motoric or phototactic activity of *A. mellifera*.

How diverse are *Lactobacillus kunkeei* isolates from honeybee midgut?

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Lactic acid bacteria (LAB) have been frequently used as probiotics in human and animal health. It has been reported that LAB strains obtained from honey bees colonies may present probiotic properties, as the inhibition of pathogens growth such as *Paenibacillus larvae* and *Ascosphaera apis*. Among those LAB, *Lactobacillus kunkeei*, has been reported as a dominant member of the native midgut microbiota. Biochemical and morphological characterization of those isolates showed differences among them. Since attributes associated with probiotic properties are strain-specific, it is therefore necessary to characterize different strains belonging to the same species.

The aim of the present work was to evaluate the genetic diversity of *L. kunkeei* isolates obtained from honey bee midguts, and evaluate the presence of different genotypes. DNA

from 55 *L. kunkeei* isolates was extracted and used as a template for genetic analysis through Rep PCR. DNA fingerprinting based on this technique is a simple and fast method to differentiate among closely related bacterial strains. It shows a good reproducibility and has been successfully applied for the classification and differentiation of strains of many Gram positive and negative bacteria, including *Lactobacillus* species. Primers BOXA1R and ERIC1R/ERIC2 were used.

A high genetic diversity was found between isolates, and at least 4 genotypes were found. Different genotypes can coexist in the same colony, even more, in the same bee. We are currently examining whether there is any correlation between inhibition of *P. larvae* by *L. kunkeei* and the different genotypes.

Use of drugs in beekeeping in Italy

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In Italy, the application of Decree 2004/28 CE resulted in a reaction of beekeepers against law enforcement in which they saw a strong economic loss. Indeed, the cost of the drugs exceeded even three to four times that of the active principle. In this work, the regional rules of the beekeeping sector that consider European law are analyzed. In pending national law about registry of bees, each region has legislated considering medication management one of the most important problem in beekeeping.

Immune response in nurses and forager bees (*Apis mellifera*) after artificial immune challenge

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The immune system protects animals against internal and external stressors. The innate immune response is shared by many animal phyla, and is known to decline during aging. The honey bee, with its flexible aging patterns, offers the opportunity to study flexible aging of the immune system, including the reversal of immunosenescence. In honey bee, as in other insects, the key immune regulators are fat body and the circulating hemocytes (blood cells). The fat body is a production site of effector molecules, while hemocytes are involved in phagocytosis, melanization and encapsulation. The present study investigated immune response in foragers and nurses to test for social task dependent response patterns, independent of chronological age.

Nurses and foragers of the same chronological age (20-25 days) were divided into three groups, one group injected in the abdomen with lipopolysaccharide (LPS), and the second group with peptidoglycan (PGN). In both cases this was done to induce immune

response. Third group was injected with phosphate buffered saline (PBS) only and used as a control.

No treatment specific effect was detected. Nurses survived longer than the foragers. Specifically, both nurses and foragers showed similar survival patterns when treated with LPS, PGN or PBS. This could indicate that the damage and inflammatory responses are more harmful than infection responses due to bacterial cell wall components. The treatments gave also different transcriptional patterns of immune related genes; interestingly this pattern differed between nurses and foragers.

The induced fermentation of artificial diets and its effects on *Apis mellifera* bees.

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As artificial diets are an alternative for avoiding the loss of colonies in periods of food scarcity the search of diets that are efficient and palatable for bees at the same time is essential. Here, we aimed to investigate of induced fermentation on artificial protein diets and its effects on the levels of protein in the hemolymph of caged bees, compared to those who were fed unfermented diets. We caged groups of 100 newly emerged bees in different plastic cages and fed these bees for 7 days with different diets: D1 (Beebread and water); D2 (Sucrose syrup 70% v/v); D3 (sugar cane yeast, soy powder, rice powder and sugar); e D4 (D3 diet added fermented inoculum). The inoculum consists of fresh Beebread collected from the hive added 50% sucrose syrup (v/v). This admixture was left in a stove for 28 days for fermentation. In days 0 and 7 of the experiments, we randomly removed 10 bees per cage for hemolymph extraction and analysis. The total protein concentration in the hemolymph was measured according to Bradford (1976) and the qualitative profile of the samples was verified in polyacrylamide gels. Diets D1, D3 and D4 were efficient in promoting protein synthesis. The densitometry results indicate that bees fed D4 present higher concentration of Vitelogenin, a storage protein when compared to D3 ($p=0.0027$). We conclude that both artificial protein diets are efficient regarding the protein levels, but D4 presents a higher impact on the production of Vitelogenin.

Gene expression in honeybee (*Apis mellifera*) exposed to acaricides and fungicides

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The Carniolan honey bee, *Apis mellifera carnica*, is Slovenian autochthonous breed. In recent years, Slovenia, similarly to the rest of the Europe, recorded 15-35% annual loss

of bee colonies due to mortality of bees. One of the possible reasons for this could be the exposure of bees to residues of insecticides, acaricides, herbicides and fungicides, which have been found in honeybees and hive products. Acaricides are applied by beekeepers to control ectoparasitic mite *Varroa destructor*, the most serious pest of honeybee colonies in Europe, whereas the most abundant and common agricultural chemicals found in honeybee are fungicides, which may enter the bee and hive products when applied to nearby flowering crop and fruit plants. Acaricides and fungicides are not highly toxic to bees alone, but they may act in combination and lead to higher honeybee mortality. In our study we investigated the molecular response of different development stages of honeybee workers (larvae, pupae, adult) exposed to a combination of acaricide and fungicide *per os*. We examined the expression of several genes involved in immune response, detoxification and development by quantitative RT-PCR. We observed that combination of acaricide and fungicide leads to a synergistic effect on several of the immunity-related, detoxifying and development genes. We also found that the acaricides and fungicides, solitarily or in combination, have important impact on gene expression in different development stages of honeybee worker.

The paratransgenic potential of *Lactobacillus kunkeei* in the honey bee (*Apis mellifera*)

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The honey bee *Apis mellifera* is a domestic insect of high human value both as a major crop pollinator and as a model animal in several fields of scientific research. However, during the last decade honey bees are faced with massive mortality and the health of colonies appears to decline worldwide. The reasons are debated but a combination of several factors including parasites, virus, pathogens and pesticides are believed to be involved. Paratransgenesis is a *Trojan horse strategy* where symbiotic microorganisms are used to deliver effector molecules that can prevent pathogen development. Here we present the first steps involved in the development of a paratransgenic method in honey bees.

The fructophilic bacterium *Lactobacillus kunkeei* was shown to dominate the honey bee midgut after screening on selective growth medium for lactic acid bacteria and was hence selected as a paratransgenic candidate. We found that *L. kunkeei* fulfills the three criteria needed for paratransgenesis: (i) it is genetically modifiable, (ii) the modified bacterium has no adverse effect on honey bee health or survival and (iii) survives together with the normal microflora upon reintroduction. Further development of the method could provide us not only an important means to fight honey bee diseases but also a genetic tool to deliver proteins and enzymes for the study of functional honey bee genetics.

Comparison of different pollen substitutes for the feeding of artificially reared bumble bee (*Bombus terrestris*) colonies

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In bumble bee colonies pollen is the only protein source for larval feeding and adults consume it to develop their glands and ovaries. Pollen deprivation leads to a poor development of the bumble bee colonies, due to the death of larvae and the reduction of longevity and reproduction in adults.

In bumble bee artificial rearing, fresh frozen pollen is the most used protein source, giving the best results in terms of colony development and queen size and reproduction. Dried pollen is also used in artificial mass-rearing.

We tried different natural protein sources, as possible alternatives for the nutrition of artificially reared *Bombus terrestris* colonies. Colonies were fed on fresh frozen pollen until the emergence of the first brood, then the feeding was shifted to the pollen substitutes.

We tested four different commercial pollen substitutes used for honey bees colonies during periods of pollen shortages (Calcalar®, Melissokomiki®, Candipolline® and Feed Bee®) and two natural protein sources (yeast and chestnut flour) mixed with sugar solution, in comparison with fresh frozen pollen administered in two different ways: honey bee pollen pellets alone or mixed with sugar solution.

None of the tested pollen substitutes allowed the development of bumble bee colonies, the best results being obtained with yeast and Feed Bee®. No differences were found between the two kind of fresh frozen pollen, in terms of colony development. Further experiments will be conducted with a mixed diet, composed by these two substitutes and fresh pollen in different percentage.

Diapause survival and colony initiation in artificially reared colonies of bumble bees (*Bombus terrestris*)

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Queen diapause and colony initiation are the most critical moments in bumble bees biological cycle, and they represent the main obstacles in bumble bee artificial rearing.

The weight of queens is one of the main determining factor in the survival to hibernation and post diapause performances. We measured diapause survival and egg-laying success of artificially reared *Bombus terrestris* queens, basing on their pre-diapause weight. As expected, less-weighted queens survive in a significantly minor number to the diapause regime (3 months at 5°C). Queens' weight have no effect on the deposition rate, but queens

with the highest weight lay significantly more egg cells.

Among the different methods of colony initiation, the use of a male pupa is strongly recommended for queen stimulation. We compared the use of a male pupa against that of a queen pupa. The number of egg laying queens do not differ between the two groups, but the amount of egg cells and of developed larvae is higher in the “queen pupae” group. Possible explanations to this result can be found in the bigger size of the queen pupa, and in the destructive behaviour of adult males at their emergence from the pupa.

Results of catches by sticky traps to detect honey bee parasitoids in Piedmont (Italy)

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Apis mellifera parasitoid attack has proved to be more widespread and significant than previously thought, related to newly-discovered roles played by Phoridae family species. Not only has bee decline in North America and *Apocephalus borealis* (Core et al, 2012) been linked, but also facultative parasitoid *Megaselia rufipes* has been detected on honeybees with deformed wings (Dutto and Ferrazzi, 2014) in the Italian northwest (Piedmont).

Senotainia tricuspis, the primary honeybee parasitoid in southern and Central Europe and north Africa studied since 1949 (Simintzis, 1949) and known to cause particular harm in central and southern Italy, is now considered associated with honeybee die-offs in many areas. In fact, reports by some beekeepers using sticky traps to catch this dipteran have suggested the species has a widespread presence. To assess the true incidence of this and other parasitoids, sticky traps were deployed in four rural Cuneo province municipalities (Piedmont). Specifically, white, yellow, and grey traps were placed in the roof fronts of hives located in different environments (near a river, a wood, a kiwifruit orchard, and grazing lands). Traps caught species belonging to the Calliphoridae, Sarcophagidae and Tachinidae, *Panorpa*, Phoridae, Tabanidae, and Aphididae taxa. The principal species caught were honeybees, with a significant difference compared to other taxa. Significant differences have been found also in the catches with adhesive strips of different colours. *S. tricuspis* was undetected, implying its absence in the wide area sampled. However, caution is advised when interpreting reports, as this species can be confused with other Sarcophagidae.

Usage of iodophor disinfection in beekeeping practice

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Honeybee colonies are under infection pressure of microbial, viral and parasitic pathogens. The pathogens can cumulate in tools and in beekeeping equipments in endemic areas with bee diseases. When infection pressure grows, it could be main reason of colony losses. Beekeepers can prevent bee diseases by common hygiene practice, e.g. periodically cleaning and disinfection of equipment.

Iodine is biogenic element in the nature. The traces of iodine are present in honey also. Iodophor disinfection is effective against a broad spectrum of microorganisms. Using of products with iodophor active substance is common in animal welfare. However, using of iodophor disinfection in beekeeping practice is not well known.

We performed *in vitro* experiment of iodophor disinfection against spores of *Paenibacillus* larvae. Then, we tested *in vitro* effect of iodophor disinfection on adult bees with tarsal and oral toxicity test. We have applied the iodophor disinfection to wooden hives. Treated hives were left on the fresh air for one month. Honeybee colonies were established in this treated hive boxes. Moreover, we analyzed honey from the iodophor treated hives.

Viability of *Paenibacillus* larvae spores were decreased after incubation with iodophor solution from 1×10^6 to 1×10^4 . The oral and contact toxicity of iodophor to bees wasn't detected. The quantity of the iodine in the honey taken from treated hives was the same as in the control honey samples.

The iodophor disinfection can be to use for periodically and prevention cleaning in beekeeping practice.

Arsenophonus sp. an endosymbiont of honey bees, *Apis mellifera*

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Arsenophonus is a diverse bacteria genus that includes different stages of association with various insects ranging from facultative to obligatory and from parasites to beneficial. These stages are related with the way of transmission to the offspring (e.g. obligatory endosymbionts prefer transovarial transmission). To shed light on the potential influence of such endosymbionts on honey bee health, we here investigate the passing route of the bacterium *Arsenophonus* sp. from *Apis mellifera* queens to their progeny.

Seventeen honey bee queens were screened with routine PCR and primers obtained from Duron et al., 2008. Five queens were found to be positive for this bacterium. The ovaries and their respective eggs were also screened to test if the bacteria have a transovarial or transovum (passing through the egg surface) transmission. Two groups of 10 eggs

each, surface and non-surface sterilized, were tested using PCR. *Arsenophonus* was not detected in the queen ovaries but in both groups of eggs. Since, it was more predominant in the non-sterilized eggs compared to the sterilized ones, this suggests transovum transmission. Our data therefore indicate a facultative stage of *Arsenophonus* in honey bees. Nevertheless, given the key role endosymbionts can play in other invertebrate systems, *Arsenophonus* sp. should be investigated in more detail to improve our understanding of honey bee health.

Relation between protein diets, protein levels in the haemolymph and honey bees health.

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The fitness of bees depends on several factors, among which quality of diet and the presence of parasites. Adequate supplementation or replacement of pollen is necessary for maintaining healthy honey bee colonies during periods of lack of pollen. We carry out two trials with caged bees, in one we compared how protein supplements elaborated with free amino acids, with a soy based diet and pollen influence protein stores within individual honey bees and in the second we tested the effect of natural and non-natural protein diets on haemolymph proteins titers of honey bee and *N. ceranae* spore abundance. The results show that the diets were metabolized in different way by bees. The supplement with free aminoacids promoted more protein in the hemolymph but less storage of protein in fat bodies at 27 d-old; and the survival of bees was lower than the others treatments. In the second trial, when bees were parasitized with *N. ceranae* died before and the survival was significantly lower at fourteen days post-infection when fed with substitute than fed with bee bread, despite these had more *N. ceranae* abundance. The overall results suggest a connection between diets and physiological condition of honey bee. Furthermore the protein levels in the haemolymph affected the tolerance to *N. ceranae*; consequently the negative impact of the parasite on host fitness is not associated only with the level of infection.

Natural plant extract (HO21); is effective against *Paenibacillus larvae*, the causal agent of American Foulbrood disease of honeybees.

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Paenibacillus larvae is the causative agent of American foulbrood (AFB). This disease causes significant economic losses to beekeepers, because it is the most harmful pa-

thology that affects larvae of the honeybees. Spores of *P. larvae* are the main vectors for the spreading of the disease. In infected hives, spores can be found in brood, honey, wax, pollen and hive walls. Much effort has gone into controlling this disease, including the use of preventive and curative treatments with antibiotics and other antimicrobial substances. Unfortunately, the extensive use of antibiotics leads to an accumulation of residues in beehive products (especially in honey), decreasing their quality and making their marketing more difficult. In the present work was evaluated the effect of HO21, a natural plant extract with antimicrobial properties, against *P. larvae*. *In vitro* antimicrobial activity of HO21 was evaluated by the disk diffusion method. Minimal inhibitory concentration (MIC), minimal bactericide concentration (MBC) and their antimicrobial selectivity were determined on J agar. HO21 had inhibitory effect on the growth of spores (MIC: 0,9 mg/ml, MBC: 1,5 mg/ml) and vegetative cells of *P. larvae* (MIC: 0,03 mg/ml, MBC: 0,08 mg/ml), was selective in its antimicrobial activity and proved to be innocuous for larvae of *A. mellifera* grown *in vitro*. HO21 is a potential therapeutic alternative against AFB, would reduce the use of chemicals and antibiotics in the production of honey.

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Sinergistic Strategy to Control Varroa in Tenerife Island

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Honeys from Tenerife just got the Protected Designation of Origin. Environmental conditions in our Island offer great diversity of flora with many endemic species. However, these same conditions make Tenerife be called “Island of Eternal Spring”, allow colonies breed around year, but unfortunately, varroa can go on reproducing continuously. So a Integrated Pest Management to control varroa has been proposed by veterinarian services of APITEN (Beekeeping Association in Tenerife). The principal objective will be avoid Critical Phase after harvest. The basic sanitary calendar pays special attention to adapt therapeutic treatment to environmental conditions and timing of blooms, the multiplication methods and the supplied feeding.

This IPM will be a synergistic strategy because, at the same time, we will try to control varroa and improve good practices in beekeeping. So, honeybee colonies will be able to reach adequate performance, vigor and tolerance to diseases and beekeepers will increase productivity and save time and money.

Finally, in this sense, it is necessary to consider if the grants for beekeepers in European Union, based on the number of hives, is according to Farrar's rule. A major European coordination of Vets, Beekeeping Technicians and Researchers would be convenient to share criteria, experiences and participate in an European Experimental Network to control Varroa.

Efficacy of heat treatment to inactivate *Paenibacillus larvae* spores in the processing of beeswax foundation.

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American foulbrood (AFB) is an infectious disease that affects the larvae or pupae stage of the honey bee. The infection can be transmitted to larvae by nurse bees or by spores remaining at the base of a brood cell. The spores of *Paenibacillus larvae* can survive in products such as honey, wax or dry larval scales and in the environment for 3 to 10 years and as purified spores even more than 70 years. In order to reduce the risk of AFB disease transmission, caused by the bacteria *P. larvae*, inactivation of the spores from bee products is a necessity. The objective of this study was to investigate how a thermal treatment applied at different times can diminish the spores without antibiotic. We obtained spores from *P. larvae* strains isolated from diseased larvae and cultivated on Brain heart Infusion agar. After several heat treatments from 80 °C up to 121 °C at different times we observed the inactivation of viable spores. So, it is necessary high temperatures or long periods to inactivate effectively the spores during the beeswax foundation.

Assessing hygienic behaviour of *Apis mellifera* L., populations from Madagascar and Réunion Island.

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Apis mellifera L. workers are able to recognize diseased, damaged or dead brood inside capped cells and removed them through Hygienic Behaviour (HB). Efficient HB of workers increases resistance to various diseases, such as varroaosis. HB of colonies is a highly heritable trait, and has been extensively selected in European and American honeybee populations.

Varroa destructor has been detected in Madagascar in 2010. Since, it represents a serious threat to honeybee populations from the South West Indian Ocean islands. These populations have never been selected for hygienic behavior.

The objective of our study was to evaluate the capacity of honeybee colonies from Madagascar and Reunion populations, still free of the varroa mite in 2013, to withstand the potential arrival of the mite. Tolerance ability was indirectly investigated by measuring variation and distribution of the HB trait within and among populations.

In Réunion, recording of the removal rate of freeze-killed brood within 48h was

performed for a total of 220 colonies from 12 apiaries. Tests were repeated twice, 15 days apart.

In Madagascar, the pin-killing brood method was used in two regions (Ambositra and Morondava). A total of 200 colonies from 20 apiaries were tested and cleaning rate was recorded 6h after perforation. 75 % of the colonies were tested twice, 15 days apart.

This work would help beekeepers to pre-select hygienic colonies and is a first step to develop future breeding programs.

Structural characterization and antimicrobial activity of biosurfactant obtained from *Bacillus pumilus* against *Paenibacillus larvae*, the causative agent of American Foulbrood

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A biosurfactant producing bacterial strain was isolated from the motor oil contaminated soil sample from warehouse, India and was identified as *Bacillus pumilus*, DSVP18 (NCBI GenBank accession no. GQ865643) by 16S rRNA gene sequencing. When Potato peel was used as the sole carbon and energy source for biosurfactant production using *B. pumilus*, a product yield of 3.2 ± 0.32 g/l was obtained. Biosurfactant obtained, reduced surface tension value of the medium from 70 to 28.7 mN/m and was stable over a wide range of temperatures (4-120 °C), pH (4-12) and salt concentrations (3-15%). Chemical composition of biosurfactant showed it to be lipopeptide in nature with 14.7% protein content and 18.0% lipid content. HPLC purified product was characterized by Fourier transform infrared spectroscopy, showed that it was a protein-bound lipid thereby imparting them special properties. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometric and nuclear magnetic resonance studies confirmed its lipopeptide nature. The antimicrobial potential of biosurfactant against *Paenibacillus larvae*, the causative agent of AFB was investigated. *In vitro* growth inhibition assays revealed that the biosurfactant exhibited antagonistic effects on *P. larvae* growth. These results indicate that the biosurfactant isolated from the *B. pumilus* could potentially be employed as a new biological agent to control American Foulbrood disease of honeybee.

***Paenibacillus larvae* protease as a virulence factor in honeybee larvae infection**

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Paenibacillus larvae (PL) is a causative agent of American Foulbrood, a severe disease affecting larvae of *Apis mellifera*. During the growth and sporulation processes PL produces proteases that could be involved in the degradation of larval tissues. Little is known about growth kinetics of Argentinean PL strains in broth, as well as virulence factors secreted by PL during larval infection. In this work, growth curves are carried out to determine CFU/mL, the total secreted proteins and the secreted proteases, in order to analyse whether the secretion/production of proteases is regulated by a quorum sensing mechanism. In a second step, essential oils are tested as regulators of that secretion/production of proteases, and/or inhibitors of the quorum sensing. Six PL strains were inoculated in J broth and incubated at 37°C with shaking (170 rpm) for 72h. Aliquots were taken at different times to determine CFU/mL, quantify total secreted proteins by Bradford, test proteolytic activity on skim milk agar, and evaluate the presence of metalloprotease by gelatin zymography +/- EDTA (metalloprotease inhibitor). The bacterial culture went through exponential growth from hour 16 to 40, followed by a 2-4 h stationary phase and a quick death phase. The highest accumulation of total secreted proteins was registered between 67-72 h (0.0013 µg protein/CFU). Proteolytic activity was manifested, and the presence of metalloproteases in a 60 KDa band corroborated by zymography. These results suggest that Argentinian PL strains secrete metalloproteases that may act as virulence factors during *A. mellifera* larvae infection.

Non-conventional molecules to control of *Paenibacillus larvae*, causal agent of American foulbrood

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American foulbrood (AFB) is a serious disease that affects the larvae of honeybee *Apis mellifera*. It is caused by *Paenibacillus larvae*, a resistant spore forming bacterium, which remains viable through long periods of time and survives environmental adversities. This plague is usually controlled with antibiotics which could leave toxic residues in honey and by-products. Laboratory and field trials were conducted to evaluate the effectiveness of non-conventional molecules in the prevention and control of AFB in honeybee colonies.

Twelve compounds were screened in order to test their antimicrobial activity against *P. larvae*. Lauric acid and menadione, an innocuous saturated fatty acid and a liposoluble vitamin, were selected to continue the study. The antimicrobial activity of the molecules against ten *P. larvae* strains was determined by the broth microdilution method. Lauric acid minimal inhibitory concentration (MIC) mean value was 20.31 mg mL⁻¹ and the MIC menadione mean value was 2.145 µg mL⁻¹. Toxicity to adult honeybees was tested by the complete exposure method. Non mortality was registered when using quantities up to 440 µg and 1500 µg of menadione and lauric acid, respectively. The results obtained in the present study contribute to the knowledge of the use of alternative natural compounds to control AFB in the apiaries. This way, toxicological risks and other undesirable effects, such as resistance due to the indiscriminate use of antibiotics, would be avoided.

Examination of winter debris as a tool for the control of American foulbrood

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American foulbrood (AFB), caused by the spore-forming bacterium *Paenibacillus larvae* (*P. larvae*), is the most serious and widespread brood disease in honey bees (*Apis mellifera*). The clinical examination is still considered the gold standard for the diagnosis of AFB but it does not enable the detection of colonies with asymptomatic infections.

Debris collected on the hive bottom trays can be a good indicator of the presence of *P. larvae* infection in the colony.

The aim of this work was to study the relationship at the colony level between the number of *P. larvae* spores in the winter debris and the probability of developing the disease in the following spring.

Winter debris collected from 351 hives belonging to 17 apiaries in the Emilia Romagna Region (Italy) were examined. Each colony was then clinically monitored monthly from March to June in the following spring.

During this period the disease evolved in only one out of the 154 colonies (0.6%) in which the presence of *P. larvae* spores in the winter debris had not been recorded. Instead the disease was observed in 19/25 (76%) colonies with a number of *P. larvae* spores $\geq 100,000$ CFU/g and a total of 44% of colonies with a number of spores $\geq 1,000$ CFU/g developed symptoms of AFB.

This study showed that there is a close correlation between the load of *P. larvae* spores in the colony's winter debris and the probability of developing the disease in spring.

Monitoring the winter debris could be a helpful tool for reducing the impact of AFB.

Rapid identification of differentially virulent genotypes of *Paenibacillus larvae*, the causative organism of American foulbrood of honey bees, by whole cell MALDI-TOF mass spectrometry

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Infection with *Paenibacillus larvae* is lethal for honey bee larvae and may lead to loss of the entire colony. Of the known ERIC genotypes (ERIC I – IV) of *P. larvae*, only ERIC I and II are frequently found in the field, whereas ERIC III and IV are only available as historical isolates. Genotypes ERIC I and II differ significantly in virulence and the course of the disease on the larval level is more accelerated after infection with ERIC II strains allowing nurse bees to remove diseased larvae more efficiently. For this reason the lead clinical symptom, conversion of capped larvae into ‘ropy mass’, is less frequently found than after infection with ERIC I strains bearing the risk of false negative diagnosis. In this study, the potential of matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry for the discrimination of *P. larvae* genotypes ERIC I and II was explored on the basis of a comprehensive set of isolates. Using commercial software and a reference database constructed from field and type strains, ERIC I and II genotypes of all field isolates could be unambiguously identified on basis of mass spectra. Statistical analysis showed that the genotype is the main determinant for the spectral phenotype and MS-based ERIC-type determination is robust against sample selection. Furthermore, analysis of samples from Canada and New Zealand showed that distribution of ERIC II is not restricted to Europe as previously assumed. We suggest adding ERIC I and II genotype isolates as type-specific reference spectra for use in routine diagnostics.

Friends or foes in the honey bee midgut? Interactions between the parasites *Nosema ceranae* and *Crithidia mellificae*

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The scarcely studied trypanosome *Crithidia mellificae* and the almost ubiquitous microsporidian *Nosema ceranae* are both midgut parasites of honey bees and can be widely distributed in host populations. However, the nature of interactions between these two parasites is poorly understood and can potentially range from synergism to antagonism at the individual worker super-organism colony host levels. Here, we aim to better understand the interactions between these two gut parasites by quantifying infection loads of individual workers and colonies. In April 2011, adult honey bee workers were sampled

from 15 apiaries across Switzerland (4 colonies each) and both parasites were diagnosed using routine PCR of individual workers (N=96 from 4 colonies of a single apiary) and pooled colony samples (N=60 workers per colony). Whereas 35.4% of pooled colony-level samples were infected by both parasites, only 2.1% of individual workers were co-infected. This suggests potential differences in parasite aggregation between the two species, or even inter-specific competition, and therefore highlights the need to better understand drivers of *C. mellificae* and *N. ceranae* distributions both at individual and colony levels.

Population genetics and conservation

Are Pyrenean bumblebees (Hymenoptera, Apidae) declining?

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Species richness of flower visiting insects has declined in past decades and nowadays the ecosystem service they provide by pollinating crops and wild plants is threatened. Bumblebees are vitally important pollinators and play a key role in natural and agricultural ecosystems. Current drastic decreases in bumblebee communities, mainly due to anthropogenic actions, have been detected in some North American, Asian and European areas with potential consequences for the stability of their ecosystems and crops yield. Given the aforementioned decline and the high bumblebee biodiversity that holds the Iberian Peninsula, an assessment of the extant species richness and conservation status, especially in orophile environments, is urgently needed.

We aim to compare present and past distributions of the Pyrenean bumblebees using historical survey data and recent collection campaigns (2006-2012). The Pyrenees possess a high biodiversity of bumblebees, as Euro-Siberian, Mediterranean and alpine faunas coincide there.

A total of 26 species and 35 subspecies have been found in the recent samplings. Four species previously present were not observed, one of them considered as Vulnerable in the Spanish Red List. With the exception of a pair of species, a loss of abundance seems to be the rule, especially in 13 out of the 26 species found. At the same time, a more orophilous tendency has been observed in 21 species and 24 subspecies. Our results reinforce the need to develop new protection programs and more restrictive conservation measures of bumblebee populations, species and their habitats.

This study has been financed by the Project Biobombus CGL2012-34897.

Exploring genetic diversity in *Paenibacillus larvae* isolates from the United States using a multilocus sequence typing (MLST) and phenotypic characterizations

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Paenibacillus larvae is a Gram positive bacteria and the causative agent of the most widely fatal brood disease of honey bees, American foulbrood (AFB). A total of thirty two independent *Paenibacillus larvae* field strains from various geographical origins in United state and five reference strains were investigated for genetic diversity using multilocus

sequence typing (MLST). This technique is known to be a powerful tool for epidemiological studies of pathogenic bacteria and is widely used in genotyping assays. For MLST, six housekeeping gene loci were studied, their profiles consisting of *ilvD* (dihydroxy-acid dehydrogenase), *tri* (triosephosphate isomerase), *pur* (phosphoribosyl-aminoimidazole-carboxamide), *rpoB* (rpoB DNA-directed RNA polymerase subunit beta), *recF* (DNA replication and repair protein) and *gmk* (guanylate kinase). Previously, ERIC type DNA fingerprinting was applied to these same strains and the data showed that almost all strains were ERIC I. Our data highlights the intraspecies relationships of *Paenibacillus larvae* and the potential application of MLST methods in enhancing our understanding of epidemiological relationships among bacterial isolates of different origins.

Differences in induced defense reactions of honeybee subspecies

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To identify intraspecific variation the laboratory model for determining the physiological state of 100 bees taken from the hive has been developed. When the cage content in a laboratory the initial stage of protective responses development induced by the action of bacterial preparation bitoxibacillin various concentrations (0.5% and 0.01%) has been observed in bees. The honeybee subspecies *Apis mellifera mellifera* L. and *Apis mellifera caucasica* Gorb. of several hives from different apiaries have been used for the experiments. At a concentration of 0.01% in *A.m.mellifera* reparative processes prevail over destructive, and at a concentration of 0.5% the level of many processes decreases in accordance with principle of protective braking and reactions providing the regenerative processes begin to be activated by the end of day. In bees *A.m.caucasica* these processes are delayed for a day. In bees of both subspecies an increase of phenoloxidase activity has been observed with increasing of the biopreparation concentration that distinguished this enzyme from other parameters of defensive reactions. At the same time, *A.m.mellifera* outnumbered *A.m.caucasica* in speed and level of protective reactions development. We assume that in *A.m.caucasica* restructuring in the power saving mode and redistribution of internal resources occurs with some delay to the end of the first day. Obtained data largely determine the vulnerability of these bees in the climatic conditions of central Russia and its northern regions and a higher resistance of *A.m.mellifera*.

Wing venation teratology in European honey bees.

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Honey bee (*Apis mellifera* L.) wing venation is quite distinctive and the resulting pattern is currently used in subspecies discrimination. In recent years the forewings of worker bees sampled in *A. m. mellifera*, *A. m. ligustica*, *A. m. carnica*, and hybrid colonies were examined and various abnormalities - due to the presence of both supernumerary and defective veins - were observed in about 4% of wings. The supernumerary veins were considered as present only if an evident vein length could be detected, while slight thickenings were ignored. Most colonies did not show any teratology in wing venation, while a few ones provided several workers with abnormal wings; in these cases, some individuals showed two or more abnormalities. Spurs of various length protruding from the standard veins were the most frequent abnormalities observed; among them an adventitious distal abscissa of the *2rs-m* crossvein and the extension of the *Rs* vein from the marginal cell distal end were rather common. In some cases two opposed spurs tended to join or a single vein branched, thus defining an open or even closed supernumerary cell. In defective veins the missing stretch varied considerably in length so as in a few cases two contiguous cells merged more or less completely. Wing teratology should be taken into account when using wing venation patterns for the morphometric distinction of honey bee subspecies and data from abnormal wings should not be acquired, especially if automated procedures are used.

Intraspecific bumblebee diversity: a case study of *Bombus pascuorum* (Hymenoptera, Apidae) in the Iberian peninsula

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Bumblebees (genus *Bombus* Latreille, 1802) are important pollinators of both wild vegetation and agricultural crops globally. Given the current trend of pollinator disappearance, knowledge on the geographic variation and diversity provides basic biological information for conservation and utilization of the resources provided for pollination. Herein we examined genetic divergence among subspecies of the common carder bumblebee *Bombus pascuorum*. We obtained mitochondrial sequences (barcoding) and microsatellite data from individuals collected across Spain corresponding to five morphological subspecies. These subspecies inhabit different areas although some overlapping occurs among them. Mitochondrial sequence data has resulted in limited resolution power in relation to subspecies discrimination. Furthermore analyses of microsatellite data with a Bayesian clustering method has been used to detect gene flow among the subspecies of *B. pascuo-*

rum. We aim to test the hypothesis that divergent phenotypes may be an example of phenotypic plasticity within this species.

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Genetic diversity of the endemic Iberian bumblebee *Bombus terrestris lusitanicus* (Hymenoptera, Apidae)

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The Iberian Peninsula harbor 39 bumblebee species and 56 subspecies constituting a rich and diverse set of social bees with high biological interest. Some of these subspecies as *Bombus terrestris lusitanicus* are widely distributed over the Iberian Peninsula but their populations may become isolated due to habitat fragmentation. We hypothesize that population genetic factors such as a reduced effective population size and limited gene flow between populations located in fragmented habitats, may result in a reduction in genetic diversity with negative consequences, especially intensified in bumblebees because of their haplodiploid system. Here we analyzed *Bombus terrestris lusitanicus* populations with microsatellite data in order to test the aforementioned hypothesis. We also discuss the results in relation to the conservation status of the endemic subspecies given the introduction of other subspecies (*Bombus terrestris terrestris*) for commercial companies and its wide use for greenhouse pollination purposes.

This study has been financed by the Project Biobombus CGL2012-34897.

Antifreeze proteins (AFPs) and cryopreservation of honey bee spermatozoa: aiming on improvement of currently used cryopreservation protocols

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Spread of varroa mite result in pollination service deficit and economical losses. A great disadvantage in the use of miticides has repeatedly been described. Success in the use of selective breeding methods (breeding of Varroa Sensitive Hygiene bees) was recently achieved. These methods rely on the instrumental insemination of honey bee queens. Therefore, further improvement of protocols for long-term storage of honey bee spermatozoa is of great interest. Cryoprotective agents (CPA) used in cryopreservation of sperm prevents ice formation during freezing, thus preventing sperm from ice damage. However, the fertility of drone sperm cryopreserved with CPA is often unexpectedly low. Recently, the toxicity of two frequently used CPA (dimethyl sulfoxide and ethylene glycol) was reported. It is important to introduce non-toxic CPA in honey bee sperm cryopreservation.

Antifreeze proteins (AFPs) were isolated from many organisms living in cold polar regions. These proteins have unique ice-binding affinity and can effectively inhibit ice crystals growth, so depress the freezing point of body liquids in polar organisms. AFPs can inhibit ice recrystallization, thus preventing thawed cells from mechanical damage caused by ice regrowth. As reported by others, AFPs can stabilize cell membrane organization during freezing. However, to our knowledge, there are no reports on the use of AFPs for honey bee spermatozoa cryopreservation despite studies in the fish, bull, ram and chimpanzee. These studies have reported improved sperm quality after cryopreservation with AFPs. In our research we are aiming on possible improvement of honey bee spermatozoa cryopreservation protocol with the use of AFPs.

Morphometric analysis of *Apis mellifera sahariensis* populations from Algeria

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The study of morphometric characters of honeybees is one of the steps of conservation program diversity. The objective of this study is to determine the morphometric characteristics of *Apis mellifera sahariensis* of south regions in Algeria to determine the purity of bee colonies. Honeybees are sampled from 92 colonies in 2008-2009, 42 colonies Tellian race divided between the coastal zone and the Tell Atlas and 50 colonies of the Saharan race from the steppe and Saharan zones. Reference samples are considered to be 14 colonies Tellian bee colonies and 9 of the Saharan race from Morocco and 9 colonies of *Apis mellifera iberiensis*. The study of 38 morphometric characters of bees sampled in Algeria has set standards for the average 38 of them measured. The principal component analysis (PCA) has separate populations of the bee Saharan studied in 5 distinct groups. These populations are compared with other populations of Algerian, Moroccan and Spanish. The PCA has revealed the existence of three distinct groups. The first group includes the Iberian bee colonies. In the second group there are still two subgroups, one set consisting of samples of the bee subspecies *A. m. intermissa*, regardless of Algerian or Moroccan origin, and *A. m. sahariensis* of Algerian origin, except for bees locality Skhoune (Ain Sefra), the other formed by *A. m. sahariensis* Morocco.

Maternal structure of Iberian honey bees inferred from whole mitochondrial genomes

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The maternally inherited mitochondrial DNA has been the marker of choice for as-

sessing Iberian honeybee variation, particularly the intergenic tRNA^{leu}-cox2 region. The data generated by massive sampling of this region confirmed early findings of coexistence of African (A) and western European (M) lineages, forming a south–north cline, and revealed unparalleled levels of haplotype diversity and complexity.

Accordingly, it has been suggested that Iberia served as a glacial refuge, and as a place of secondary contact between European and African lineages. While we have learned a great deal with this region, due to its high levels of variation and repetitive structure, there are evolutionary questions that can only be properly addressed using other mitochondrial regions. In this study, we used NGS technology to sequence the whole mitogenomes of 70 *A.m. iberiensis*, 7 *A.m. intermissa*, 7 *A.m. sahariensis*, and 8 *A.m. mellifera*. The data was analyzed using a phylogeographical framework to address the following questions: When did M and A lineages split? How divergent are African-derived Iberian honey bees from both African subspecies? Is there evidence of multiple range expansions of northern African honey bees? Which African subspecies is the most probable source of African genes in Iberian honey bees? The powerful data set generated in this study is providing the opportunity to decipher the Iberian honey bee history at an unprecedented resolution. At the same time, it will supply a strong scientific basis for conservation decisions, which is increasingly important in face of the escalating threats to honey bee diversity.

Fine-scale analysis of Iberian honeybee populations reveals a strong maternal structure and the presence of novel mitochondrial haplotypes

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Apis mellifera is naturally located in Europe, Africa, and Middle East. Adaptation to different environmental conditions has enabled the evolution of 30 subspecies, which are grouped into four lineages (A–African, M–western European, C–eastern European, and O–Middle Eastern). In Western Europe, Iberia naturally harbors the Iberian honeybee subspecies, *A.m. iberiensis*. Previous mitochondrial DNA surveys of Iberian populations have revealed a complex haplotype pattern due to the coexistence of two highly divergent lineages (A and M) forming a well-defined southwestern-northeastern cline. However, these surveys did not cover the whole territory of Iberia, missing an important part such as the Atlantic. To fill this spatial gap, over 711 colonies were sampled across three north-south transects. The samples were sequenced for the highly polymorphic tRNA^{leu}-cox2 mitochondrial region and the sequences were analyzed using MEGA and NETWORK. In addition to confirming the previously reported cline, our results show (i) the presence of 25 novel haplotypes (16 African and 9 European); (ii) a higher proportion of colonies belonging to the A than to the M-lineage; (iii) a low level of introgression from C lineage; and possibly a novel African sub-lineage. While providing a more complete picture of the Iberian honeybee maternal variation, this study suggests that in spite of intensive

colony management, Iberia still maintains a strong genetic structure and is virtually free of C-lineage maternal introgression. This pattern contrasts with that reported for the other M-lineage subspecies, *A. m. mellifera*, which has been threatened by introgression and replacement by C-lineage honeybees.

Morphometric study of bees in Poland. The interpretation of morphometric data using the microsatellite DNA markers.

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For the determination of subspecies of bees bred in Poland the features of the fore-wing are used. The wing vein junctions are treated as coordinates and then subjected to canonical analysis. Determination of junctions as calculation procedures are automated by a computer software. The results of this analysis are expressed as indicator of difference from the model population. Models have been developed on the basis of other morphometric traits: the width of fourth tergite, cubital index and the length of proboscis. These models have been designated for three subspecies of honeybees: Carniolan, Caucasian and European dark bee.

During the survey we found colonies that did not fit the model of the declared subspecies as well as any other developed models. It was decided to verify these bee subspecies (outside the model) on the basis of DNA markers (17 microsatellite loci). The analysis included also bees verified due to wing's image and disqualified due to crossbreeding.

There was a significant relationship between the study of bee subspecies, based on wing venation and microsatellites. The probability of belonging to the declared subspecies was the lowest in samples disqualified based on wing venation. In contrast, the highest probability, regardless of subspecies was in the samples with mismatched morphology of the wings (out of the model).

Differentiation of the honeybee subspecies races in Russia using microsatellites

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The development of molecular genetics methods opens up new possibilities in the characterization of the allele pool of *Apis mellifera* L. specie. Based on application of a variety of morphological and molecular methods at least 28 different subspecies of *A. mellifera* have been identified. The hierarchy some of the taxons is not completely clear.

The objective of this study was to evaluate the differentiation degree of *A. m. carnica*

and *A. m. carpatica* using seven microsatellite loci (A024, A88, A113, AP043, HB-C16-05, HB-THE-03, HB-C16-01). With comparison aim *A.m.mellifera* and *A.m.caucasica* bees were involved in the analysis. The genetic differentiation degree was evaluated based on the similarity coefficient (Q) calculated using the Structure software (ver.2.3.1) for the cluster number (k)=3 and 4.

The analysis showed for $k = 3$ a clear differentiation of the studied groups according to their membership of different evolutionary branches: M (*A.m.mellifera*), C (*A.m.carnica* and *A.m.carpatica*) and O (*A.m.caucasica*). The calculations carried out for $k=4$ which corresponds to the number of studied groups revealed the clear differentiation *A.m.mellifera* ($Q_{2/4} = 0,788 \pm 0,021$) and *A.m.caucasica* ($Q_{3/4} = 0,941 \pm 0,008$), whereas the corresponding values for *A.m.carpatica* and *A.m.carnica* were 0,031-0,049 and 0,051-0,099. *A.m.carpatica* and *A.m.carnica* were characterized by less differentiation degree, which was indicated by closer Q values in common clusters: $Q_{1/4} = 0,528 \pm 0,031$ and $0,788 \pm 0,031$, $Q_{4/4} = 0,341 \pm 0,027$ and $0,112 \pm 0,023$, respectively. The research results can be considered as indication that *A.m.carpatica* is most likely among *A.m.carnica* subspecies than a separate subspecies.

Genetic diversity of the endemic honeybee: *Apis mellifera unicolor* (Hymenoptera: Apidae) from Madagascar

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Apis mellifera unicolor is a tropical honeybee endemic of Madagascar. Comprehensive knowledge about its mitochondrial and nuclear genetic diversity and structuration was our main purpose. Samples were made on adult workers of 884 colonies from 76 sites of 18 regions. Enzymatic restriction (RFLP) and sequencing were used to reveal variability in the tRNA-Leu –coII intergenic mtDNA region. Seventeen microsatellite loci were used for studying the level of nuclear diversity of the populations. Three RFLP patterns were observed, among which 99.59 % of them were from the African pattern A1. Within this A1 lineage, 14 haplotypes were detected using sequencing. Only 0.41 % (3 individuals) of RFLP patterns observed was associated with an A4 pattern. Moderate genetic diversity values ranging between $H_e = 0.34$ and 0.47 were found, together with low mean number of alleles ranging from 2.47 to 3.88 alleles over all loci per population. Bayesian clustering method (Structure) assignment and DAPC separated populations into 2 well differentiated genetic clusters, and further into 5 sub-genetic clusters which matched with geographic areas. Several hypotheses are further discussed regarding to the low genetic diversity of the endemic honeybee *A. m. unicolor* in its native range.

SNP detection in French populations by whole genome sequencing

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In France, until the middle of last century the endemic populations of honey bees were represented by a single subspecies, the black bee, *Apis mellifera mellifera*. More recently, apiculturists have demonstrated an interest in using other subspecies and hybrids between *A. m. ligustica*, *A.m. caucasica*, and *A. m. mellifera*, which have been found to be more efficient producers of honey and royal jelly, and also to be less aggressive. Most population genetic studies to date in *A. mellifera* have been performed using microsatellite markers, and more recently with high density SNP microarrays. However, recent advances in high-throughput sequencing and declining costs now make population studies at the genome level feasible. We propose to study introgression and the effects of hybridisation by whole genome sequencing (Illumina™) haploid males from a large number of colonies. Sequencing of haploid rather than diploid individuals enables high confidence variant calling, reducing the need for greater depth of coverage, and therefore allowing more individuals to be sequenced for an equivalent cost. We present the preliminary results from two sequencing protocols and three SNP detection pipelines, indicating on average 95% coverage ($80\% \geq 6X$) to the *Apis mellifera* Amel_4.0 genome assembly, and the detection of a core set of more than 600 000 SNPs per individual.

Morphometrics analysis of four species of the genus *Centris*

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This study aimed to investigate seasonal variation of weight and size of four species of *Centris* (*Centris aenea*, *Centris caxiensis*, *Centris tarsata* and *Centris xanthomelaena*) throughout the year. This study was carried out in a Caatinga area, in Petrolina, PE, Brazil. Bees were collected during flower visits using an entomological net. Subsequently, bees had the intertegular distance measured using a digital caliper rule and dry weighed using an analytical scale. The total database, which included morphometric and climatic data, was analyzed using the software R version 3.0.1. A total of 420 bees was collected. *C. xanthomelaena* was the most abundant species and *C. aenea* and *C. tarsata* were the most evenly distributed species throughout the year. The weight and size of bees presented a positive correlation ($r = 0.7$, $P < 0.001$). Morphologically similar species like *C. aenea* and *C. caxiensis* presented significant differences in the measurements of weight and size ($P < 0.001$). *C. aenea* individuals presented a seasonal fluctuation of weight and size measure-

ments throughout the year ($P < 0.001$) and the Tukey's test indicated significant differences among individuals collected in the rainy and in the dry periods ($P < 0.01$). The weight and size of bees and climate data were analyzed together and the adjusted linear model showed that the weight of bees is related to size and to seasonal variation. Our results suggest that the size and weight of *Centris* bees in Caatinga is probably related to *food availability and/or food quality* variations.

Do the patterns of wing venation of workers and drones of *Apis mellifera* tell us the same history? A case study in the Iberian Peninsula.

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In the last years, geometric morphometrics of wings has been largely used as a tool to identify species, characterize genetic variability among populations, environmental stress among several other applications. In social bees, due to bias in the production of sterile females, most of the studies use workers, leaving the males as a neglected group. However, due to its importance as reproductive individuals, the use of males should be incorporated in these analyses, in order to better understand the evolutionary process and patterns found. Our aim here is to test whether a study conducted with *Apis mellifera* drones and workers from the same colonies would give us the same results, in order to validate the use of drones in morphometric and evolutionary studies of bee populations. We used one worker and one drone per colony from 711 colonies sampled in three transects along the Iberian Peninsula and also from *A.m. mellifera* and *A.m. intermissa* as representatives of the evolutionary branches that influence these populations. The results indicate the existence of an admixture cline from north to south, with northern populations closer to the M branch than to the southern populations, more similar to the A branch. Lack of structuration was also found among these populations. Our results corroborate the findings of other studies and confirm the efficiency of using drones wing morphology as a tool to evaluate populational variability.

A first approach to the morphological and molecular characterization of the stingless bees (Apidae: Meliponini) of Southern Ecuador

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Stingless bees (Apidae: Meliponini) constitute a highly diverse and abundant group of pollinators in the Neotropics, therefore playing a fundamental role in tropical and sub-tropical ecosystems. Furthermore they have socio-economic importance due to its uses in

honey and pollen production by local people. Despite a major number of threats (human land use, loss of native habitats, exotic species, climate change) there is still uncertainty about the real number of species and their populations conservation status. Here we have sampled a wide number of stingless bees species through Southern Ecuador (El Oro, Loja and Zamora Chinchipe provinces). All specimens were measured with landmark-based geometric morphometric methods and the mitochondrial cytochrome oxidase I (cox1) barcoding region was sequenced. Results confirmed that integrating morphology with DNA barcoding resulted in a successful approach to characterize these groups of bees.

Quality of honey bee queens through physical characters and colony performance

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Quality in relation to queens and drones infers to certain quantitative physical and/or behavioural characters: it is generally believed that a high quality queen should have the following physical characteristics: high live weight, high number of ovarioles, large size of spermatheca, high number of spermatozoa in spermatheca, and be free from diseases and pests. However, it is also known that the performance of a honey bee colony is the result of its queen's function as well as of the drones mated with her. These two approaches are often considered together and give a general picture of the queen production technique and selection. Here we have described the most common and well known anatomical, physiological, behavioural and performance characters related to the queens, as measured in different European countries. The presented data fit well with the findings of the same characters in the literature, and in general they support the argument for the term "quality characters". Especially for the weight of the queen, the number of ovarioles, the volume of the spermatheca and the number of spermatozoa, data per country proved its own accuracy by repetition through the years. Furthermore, we had an inside view of the sanitary conditions of the colony. This is the first step to summarize this type of diverse data for such an important issue. The knowledge acquired can be used to fill in the existing gaps in the breeding or queen evaluation systems of each country in order to facilitate standardization of methodology for comparable results.

Adaptive co-management of honey bees and crops: any resilient issue in France?

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Modern bee-keeping has progressively transformed a leisure activity into a commercial one with an increasing interaction between honey bees and crops. Three contrasting situations set up the scene in France: crop farmers provide specific nectar-rich plants to bee-keepers producing a labeled honey (i.e. wheat-lavender farming systems), crop farmers require pollination from honey bees (i.e. fruit trees or vegetable seed production systems), bee-keepers take profit of oilseed crop's nectar (i.e. raps and sunflower cropping systems). These situations lead to a shift of mental models on agriculture, from a provider of ecosystem services to a source of major perturbation on the environment (bee collapse). INRA and ITSAP are developing a collaborative research on describing and analyzing a wide set of mental models among the people concerned by the co-management of bees and crops. A first study provided a typology of mental models of a wide set of stakeholders (crop farmers, bee-keepers, politicians, environmental associations, crop firms, pesticide firms) and identified the key points where a common understanding was lacking. A participatory research leads to a common representation of the functioning of the crop farming-beekeeping socio-ecological system and to identifying several challenges to take up for a successful adaptive co-management. The options of transformation evoked, during the collaborative workshops and the companion modeling process, included involving beekeepers when designing the cropping system, co-monitoring the foraging impact on grain, seed and honey production, or adapting crop treatments and apiary management. The main challenge remains the resilience assessment of the co-evolution of two agricultural socio-ecological systems partly overlapping in space and time and which panarchy requires specific understanding. The way both hierarchical are evolving systems from field to landscape according to the multiple interrelated elements they share is discussed. The way these combined human-natural systems are interlinked in continual adaptive cycles and the cross-scale impact of ecological, sociological or economic changes is questioned.

Two New Species of *Caenohalictus* Cameron, 1903 (Hymenoptera: Halictidae) from Colombia

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Caenohalictus Cameron is a widely distributed genus in the Neotropical region, being especially diverse in the Andes of South America (Moure & Hurd, 1987; Gonzalez *et al.*, 2005). In this study describes and illustrates females and males of two species of *Caenohalictus* Cameron, 1903: *C. sabanaensis* **n.sp.** and *C. alexandrei* **n.sp.** Both species are commonly collected in the Savanna of Bogotá, a high plateau in the central region of

the Eastern Andes of Colombia. Notes on morphological variations within and among the species, as well as comments on some biological aspects and images of diagnostic structures are provided.

Diversity of wild bees (Hymenoptera: Apoidea) on a high Andean ecosystem Cundinamarca-Colombia.

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The Andes has been listed as one of the most biodiverse regions in the world and it is estimated that approximately 12% of the bees in Colombia are located in this mountain range. In Colombia the study of wild bees has focused on areas of low altitudes, our work constitutes the first inventory of bees in highland ecosystems. The study was conducted in Parque Natural Chicaque, considered a relict of Andean forest, which is located in the department of Cundinamarca, by classification of Holdridge life zones, corresponding to a lower montane forest. Two transects altitude between 2200 to 2600 were established. 3000 m long by 2 m wide, representing a total area of 6000 m² sampling. Collecting bees was conducted for two days a week between 7:00 and 17:00 hours, each week the two areas were sampled. Sampling was conducted on a total of 80 days (40 days for each zone), between October 2012 and March 2014 (800 hours total). Bees were captured primarily using an entomological net and trap euglossine bees (Apidae, Euglossini). 1035 individuals were collected from the five bee families reported for Colombia, the family was more representative Apidae with 60% of all genera, followed by Halictidae with 15%. The results of this study contrast with studies in lower altitudes, following the hypothesis that at higher altitudes decreases the diversity of bees.

Routine checks of the bacteriological quality of drone semen is necessary in instrumental insemination of queens.

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Checking bacteriological quality of semen is a routine procedure in many species but there are few records associated with *Apis mellifera* L. This study was carried out to assess the bacteriological quality of semen. Mesophilic aerobic and microaerophilic bacteria were characterized and the number of total viable bacteria per milliliter was determined in

semen samples from hives, from the apiary of INTA-FCA Balcarce, PROAPI – Argentina. Semen collection was performed according to the Laidlaw technique following good practices laboratory. Identification was carried out by routine biochemical tests and standard disk diffusion method based on the Clinical and Laboratory Standards Institute guidelines. Samples were treated with different antibiotics. Spermatozoa viability was estimated at room temperature (21°C) at initial time, one and two hours later, in pure semen (PS), diluted semen with kiev buffer (DS) and semen diluted with antibiotic (DSA). Colony-forming units (CFU) were variable from 1300 up to 15100 CFU/mL. *Staphylococcus epidermidis*, *Bacillus cereus*, *Acinetobacter Iwoffii* and *Pseudomonas putida* were identified,. All samples were sensitive with CBM Streptomycine 0,02 g/100mL and Amikacin 0,03 g/100mL. Viable counts from the samples with initial high viable counts were negative. There was no difference ($p > 0.05$) in sperm viability in PS, DS y DSA. The more effective antibiotics and concentration was different from our work performed in same apiary. It is recommended to perform microbiological test at the beginning of the mating season.

Lack of African derived honey bees on Hawaiian archipelago

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Honey bees (*Apis mellifera* L) are naturally distributed in the Old World and were introduced in America and Australia since the sixteenth century in an attempt to increase the honey production in these continents. Four subspecies were mainly imported: *A. m. ligustica* and *A. m. carnica* from east Europe and *A. m. mellifera* and *A. m. iberiensis* from west Europe. These races become adapted to the new environment and therefore considered as feral populations. From 1965 the African race *A. m. scutellata* was introduced in Brazil and since then it has hybridized with the feral colonies given rise to an out-of-control process known as Africanization. In this study we have analyzed honeybee samples for their mitochondrial variation in an attempt to detect African-derived colonies in the Hawaiian islands. Only European haplotypes have been detected thus confirming the lack of African derived honey bees on Hawaiian archipelago.

Genetic Variation in Swiss Honey Bee Populations using genome-wide SNP data

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Because of the severe colony collapses reported during the last decades as well as the continuous gene flow from introduced *Apis mellifera* subspecies, it is becoming even more important to preserve the genetic diversity in the native populations of honey bees. In Switzerland, endemic ecotypes of *A. m. mellifera* are adapted to short foraging seasons and long winters. The presence of at least three other subspecies in the country may lead to the loss of valuable combinations of traits shaped by natural selection through introgression.

Here we present a strategy to monitor the genetic diversity in Swiss honey bee populations of *A. m. mellifera* in Switzerland using a commercially available high density single nucleotide polymorphism (SNP) array. We further discuss the implications on the management of the four currently established conservation areas of *A. m. mellifera*. In particular, we like to address the following questions: How admixed are *A. m. mellifera* populations and what is the genetic diversity in these areas? How can molecular tools be used for conservation management? Data analysis will be performed using ADMIXTURE.

Managed and wild bumblebees in landscapes dominated by greenhouses

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Pollinators improve fruit and seed yield of many crops. Huelva province (Spain) is amongst the major producers of berries worldwide. To improve yields within the greenhouses, growers place commercial hives of managed bumblebee *Bombus terrestris terrestris*, which is an invasive species worldwide and a vector of pathogen spill-over from the greenhouses to the surrounding habitats. Our main aims were to assess (1) whether the managed bumblebee *B. terrestris terrestris* forages outside the greenhouses, and (2) whether its abundance, and that of the wild native bumblebee *B. terrestris lusitanicus*, relates with different land uses. Our starting hypothesis was that the abundance of managed bumblebees is positively associated with greenhouse cover in the landscape, while that of wild bumblebees is positively associated with the cover of natural habitats. Sampling was carried out in winter and spring 2014. Bumblebees were surveyed in 19 pinewoods with different cover of greenhouses and natural habitats in their surrounding 2 km radius landscape. Our preliminary results show a positive relationship between the abundance of managed bumblebees and greenhouse cover in winter but not in spring.

Preference of mason bee females (*Osmia bicornis* L.) in relation to different types of nesting material

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In 2013 four kinds of nesting material were compared. These were: grooved MDF plates (Medium Density Fibreboard), grooved plates of linden wood, plastic plates forming nesting blocks, and common reed tubes as the control. To check preferences of the mason bee in relation to different nesting materials under identical flow conditions, 4 nesting boxes were placed in 150-m intervals. The boxes have been filled with different nesting materials and 4 thousand cocoons were placed in each box.

Problems with storing the cocoons resulted in very poor hatching of bees in 2013 – about 40%. However, the percentage of hatching bees in each combination of the experiment was similar and this allowed to assess bees' preferences in relation to different kinds of nesting material.

The highest index of population growth (although very low) was noted for common reed (0.62). Population growth of mason bees reared in grooved linden wood plates was lower - 0.4; while in grooved MDF plates this index was 0.15. In the part of the experiment where the mason bees had plastic plates as the nesting material, no established cell was found. The above-mentioned results, despite insufficient hatching, allowed assessing suitability of using different nesting materials in rearing the mason bee. Common reed turned out to be the most suitable material, with linden wood plates as the second best. Other materials used in the experiment, made entirely (plastic plates) or in a substantial part (MDF plates) of synthetic materials, were tolerated much worse.

The studies are continued in 2014.

Effects of stressors on the behavior of bee pollinators

Eco-ethology of bees visitors on *Vicia faba* L. var. *major* (Fabaceae) in Algeria

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Due to their ecological key position and diversity, plant-bee relationships constitute excellent models to understand the processes of food specialisation. The purpose of this study is to define and identify the most important species of bees foraging broadbean flowers, we estimated morphological, phonological and behavioural features. We discuss the results by considering the food specialisation level of the visitor. In the studied populations (Algiers, Algeria), visiting bees belong to four different genus: *Apis*, *Andrena*, *Eucera* and *Xylocopa*. *Eucera* is foraging broad beans flowers during months of April, May. The genus *Andrena* and *Xylocopa* were found on weeds after the flowering period of beans. The two species have not a preferred type of vegetation compared to *Eucera*. The main pollinators were generalist bees such as *Apis mellifera* L. and *Xylocopa pubescens* Spinola (Apidae), and specialist bees such *Eucera numida* Lep. (Apidae). The results show that no one of the studied species, neither the specialist, nor the generalist ones, share adaptative morphological or behavioural features that may improve foraging on *Vicia faba*. However, there is a narrow synchronisation between the daily and yearly phenologies of *Eucera numida* and those of *V. faba*. This could be an adaptation of the specialist bee to its host plant. Thus, the food specialisation of *Eucera numida*, as for most specialist bees, would be more linked to its adapted phenology than to an adapted morphology.

Bee-Eaters: Pests or Alies of Bee Pollinators? Assesment Framework and Preliminary Evaluation in the Iberian Southeast (Spain)

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We present an assessment framework for the interaction between Bee-eaters (*Merops apiaster* Linnaeus, 1758; Aves: Meropidae) and bee pollinators, and a first evaluation of Bee-eater predation on honeybees in the Iberian Southeast.

We have confronted the realized or perceived damage by Bee-eaters with its potential beneficial functions regarding bee pollinators. A local estimation of honeybee predation has also been made, on the basis of direct field observations and pellets collected in bird colonies and roosts.

Bee-eaters perform some ecosystem functions and services that can benefit pollina-

tors (control of bee predators, environmental bioindication, warning against pathogens or pests). This has not dispelled the common perception that they represent a significant threat to beekeeping, rarely supported by sound assessments. On average, a Bee-eater captures $1,300 \pm 760$ bees (range 45-2,238) during its stay in the Iberian Southeast (less than the daily renewal rate of a single beehive). Except in the rare event of queen capture, or when exposed to vagrant pre-migratory flocks, it is doubtful that direct predation or induced stress can affect significantly the performance of bee colonies. Moreover, the availability of alternative invertebrate prey around breeding colonies seems to mitigate the risk of honeybee predation.

The neglected Bee-eater services could partially offset the impacts of predation and interference and change its current perception. Although Bee-eater predation on honeybees is undeniable, it seems endurable by colonies except under particular circumstances, which could be mitigated (e.g. through beehive spatial location) or compensated, since the bird's legal protection status makes it not amenable for control.

Changes in the seasonal activity of honey bee colonies over 30 years.

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The first cleansing flight dates has advanced by over 1 month during above 30 years of observations in Poland. Over the study period, the mean first cleansing flight date was 17 February ranging from 6 January to 31 March. There was a significant relationship between the date of the first cleansing flight and January to March mean temperature. The regression model suggested a 1°C increase in temperature was associated with a 4.37 day advance in first cleansing flight date.

A distinct shift in nectar flows towards the spring months has been observed in the last years. Initially, in the years 1950-1963 the tendencies for the main flow to occur earlier in the season were explained by a change in flow pattern brought about by recultivation of idle lands, decline of weeds and cultivation of rapeseed. In the recent years the weather conditions have had an impact, with very warm May in particular. Consequently, there has been a steady increase of May's contribution to the total year's gain of hive weight that reflects in honey harvest. It was 31.2% in the years 1974-1977, 36.1% in the years 1975-1982 and 37% in the years 1986-1995. In the years 1995-2003 May's importance as a contributor to gross hive weight gain reached 42.7%, and from 2003 to 2013 it ranged 40-45%. The percent contribution of June's gross gain to the total gain increased slightly but July's share in the overall gross gain substantially decreased (down to 20.2%).

The Bumble Tumble: a new Pollen Trap for Bumble Bees

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We developed a pollen trap for bumble bee hives. This device can be placed in front of the hive entrance to intercept pollen loads. Using pollen traps is common practice in studies with honey bees, however for bumble bees such investigations thus far had to be carried out by hand. Pollen foragers had to be intercepted and pollen loads removed manually; a time consuming task that furthermore can strongly influence the behavior of worker bees.

A mechanic approach to collect pollen from bumble bees is less straightforward than for honey bees. Bumble bees exhibit, in contrast to honey bees, a high size polymorphism in their worker caste. We constructed three different trap mechanisms that allow different sized foragers to pass through. The capture rates are compared under field conditions by using bumble bee (*Bombus terrestris*) colonies. Bumble bees were observed before and after passing the trap. The best trap intercepted pollen from a quarter of the pollen foragers, which is comparable to most honey bee pollen traps. We then used it in a case study comparing pollen loads collected by honey bees (N=6 colonies) and bumble bees (N=6 colonies). Preliminary results confirm the function of the developed trap mechanism under field conditions. We recommend verifying the trap performance in larger scale experiments to cover different environmental conditions.

The new developed pollen trap is a suitable tool for fundamental and applied bumble bee research. Possible applications are pollination and nutrition studies as well as landscapes ecology approaches.

Pollen supply affects the quality of honey bee drones

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The ability to ejaculate semen and the semen quality of drones reared in colonies with limited and unlimited access to pollen were evaluated. Examinations were repeated four times, during 2 seasons. Each replication used 6 bee colonies, equipped with a bottom pollen traps. Pollen traps were activated 7 days before experiment, in 3 experimental colonies only. In other 3 colonies (control ones), pollen traps were open. Queen bees in all colonies were isolated on drone combs on the day the experiment started. After 24 hours, queen bees were released. On the 23rd day after egg laying, the combs were placed in cages which were subsequently incubated at 34°C. The drones emerged during 24 hours were put in cages in the bee colonies where they remained to the end of the study. On day 15 of their life the proportion of drones evertng the copulatory apparatus and ejaculating

was determined. The semen quality of each drone was evaluated on the basis of ejaculate volume, spermatozoa concentration per 1 μ L, and spermatozoa viability. Colonies from group with limited access to pollen reared drones with smaller mass, which ejaculated in fewer numbers and released smaller amounts of semen. The drones from the two groups did not differ in the number and concentration of spermatozoa and viability of spermatozoa in the ejaculate.

Stress and protective factors in the vicinity of colonies related to physiographic diversity of the landscape, anthropogenic infrastructure and climatology.

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Failure in the management of the European honeybee *Apis mellifera* L, could be due to multiple stressors, some of them are crucial for the survival of the colony and therefore they should be considered as critical points in the hives' location. Capacity environmental foraging and changes of climatology along time may threaten the survival of the colony and compromise the profitability for the beekeeper. Knowing these stressors could allow to act on its geographical location selecting the best bee foraging area. The objective of this work is to select critical points (spatio-temporal scale) based on the protective and stress factors; such as physiographic diversity and land vegetation associated to landscape (protective factors) and some anthropogenic infrastructures (railroad track, roads, antennas and electrical lines), industrial and urban areas and intensive agriculture (stress factors). All the protective and stress factors were assessed in 15 hives of honeybee (*Apis mellifera*) located in Central Spain and an Indicator of Quality was provided for each of them. Geo-referenced data in hives and in an area which express the suitable habitat of the bee were gathered from Cartographic Base in digital format on land uses, forest coverage and anthropogenic infrastructure. Climatological variables such as monthly average temperatures accumulated, precipitation, relative humidity and wind speed and direction was obtained from the Spanish Agency of Meteorology (AEMET). Analyses were conducted using ArcGIS version 10.1 (ESRI®). Discussions about the most important critical factors to join the Quality Index and final recommendations about location were also taken into account.

Pollen quality: field and laboratory evidences of a key-role in honey bee health

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Pollen quality plays a crucial role in honey bee health and its involvement in bee

decline should be investigated deeply. The pollen may influence honey bee health not only with its nutritional qualities but also for the contamination that may occur due to pesticides application. In the nation-wide monitoring project BeeNet we found that the crude protein content of beebread collected in Autumn is negatively related to the percentage of agricultural area surrounding the apiary. Moreover, the number of pesticides found in beebread was positively related to the surface of agricultural areas surrounding the apiaries. Furthermore, the low crude protein content in beebread negatively influenced the overwintering and the annual colony survival.

At a laboratory scale, we verified the influence of pollen quality on honey bee development and sensitivity to pesticides. First, a pollen diet collected in a monocultural area negatively affected the survival of bees and increased their susceptibility to fipronil (a systemic pesticide). Secondly, we found out that exposing honey bees to sub-lethal doses of thiamethoxam in combination with a low quality diet reduced the development of the hypopharyngeal glands in young bees.

These results suggest the importance of a high-quality pollen for the health of honey bees, which might be achieved diversifying the agricultural landscape and decreasing the pesticide contamination.

European Orchard Bee *Osmia cornuta* for Pollination of Black Chokeberry *Photinia melanocarpa*

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The solitary bee *Osmia cornuta* (Latr.) have been used as excellent pollinator of almond, apricot, pear, apple and several other rosaceous fruit plants in Europe. In the last decade, Serbia has increased interest in growing black chokeberry *Photinia (Aronia) melanocarpa* (Rosaceae) variety “Nero”, primarily because the fruits are widely used for juice production. The study carried out in a black chokeberry orchard in western Serbia aimed to consider two aspects: 1) to optimize *O. cornuta* pollinating potential and 2) to reveal and enhance its field augmentation. Cocoons of *O. cornuta* were kept under special temperature conditions during the winter in order to synchronize the activity of females with the later flowering period of black chokeberry. Furthermore, hedge of *Prunus spinosa* L. was used as complementary food sources in order to anticipate the release of pollinator cocoons in the chokeberry orchard, which allowed females to initiate the nesting/foraging activity before the black chokeberry start blooming. In first week of April 2013, cocoons were placed in the orchard at an area of five ha, while the other half of the orchard was without managed pollinators. By the analysis of pollen from the *O. cornuta* nests, it was found in average 70% of *Photinia* and near 30% of *Prunus* pollen. Data showed that the augmentation of *O. cornuta* in black chokeberry orchards was twofold in relation to the number of cocoons taken outdoors. In addition, the amount of black chokeberry fruits was increased for about 15% in the orchard with *Osmia* bees.

Thin tree branches: a reduction of nesting sites for a Neotropical stingless bee

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Cavities in trees are key resources for the maintenance and the reproduction of most species of Neotropical stingless bees. *Melipona subnitida* is the most commonly domesticated social bee species in areas of Caatinga, a tropical dry forest in interior northeastern Brazil. Over 50% of the nests of *M. subnitida* are recovered in tree branches of a single plant species: *Commiphora leptophloeos* (Burseraceae). Thicker branches are preferred over thinner branches. We compared the mean circumference of branches containing nests of *M. subnitida* between the years of 1998 ($N=65$) and 2014 ($N=70$) (12 sites in the states of Rio Grande do Norte and Paraíba, Brazil). Our data showed an average decrease of 11 cm in the circumference of branches of *C. leptophloeos* between the two analyzed periods ($X_{1998}=51.5$; $X_{2014}=40.5$, $df=133$, $t=4.83$, $p<0.001$). The results suggest a restriction of nesting site availability, evidenced by the reduction in the size of the branches used by colonies of *M. subnitida*. Therefore, considering the environmental services provided by stingless bees and their importance to the local economy, policies for the conservation and management of the tree species used for their nesting in dry regions are urgently needed.

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Modelling the impacts of various pesticide effects on the honeybee colony

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Studies have shown that the sublethal effects of pesticides may have a significant effect on the behaviour or development of the honeybee (Dai et al. 2010, Schneider et al. 2012 for example) and that this may lead to adverse effects on the colony as a whole. We have been using the BEEHAVE model (Becher et al. 2014), to investigate the effects of brief periods of increased foraging mortality, reduced egg-laying and disturbed larval development on the health of the colony, with an intent to aid further pesticide risk-assessment, as it has been suggested that a more solid understanding is required before sublethal effects can be confidently included in the risk assessment (Thompson and Maus 2007). BEEHAVE is an individual-based model that includes multiple stressors acting on a honey bee colony, allowing a more realistic model of the reaction of the colony to, for example, the introduction of a pesticide to the landscape. The use of modelling in this investigation is of great value as to conduct field experiments with enough power to answer the same questions has already proven to be difficult (Cresswell 2010), and to continue the trials over long periods of time is a difficult task. The results produced show that the effects are very

dependent on the timing of the effects through the year and that sublethal effects can have a significant effect on the colony health.

Flying activity of TNT conditioned honey bees in field conditions

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Landmines present serious problem in areas involved in war activities worldwide. Without clear records, and with the impacts of weather and time, their removal becomes very complex. Buried landmines release weak odour above ground surface. These odour emissions can be detected by animals, where trained dogs are most commonly used. The development of methods for honey bee colony training to detect landmine odours in diverse conditions, including tests on the test mine-field has been carried out for several years. Conditioning of honeybee colony is carried out within the mesh tent using sugar syrup and military-grade TNT scent. Fourth day after start of the training, bees clearly recognize target odour. Conditioned honeybee colony is transferred to the test area. At the new location, conditioning with target odour and food reward has to be shortly repeated. After removal of the feeders, bees start to search for the trained odour in the surrounding. Bees demonstrate the highest flying and landing activity during morning hours (57,45 %) in comparison to noon (16,54%) or afternoon (26,01%). By offering sugar solution with TNT odour in early morning, interest of honeybee colony can be prolonged more than 15 days.

First report of lethal effect of insecticides in Neotropical bumblebee, *Bombus atratus* workers.

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In Colombia the native bumblebee, *Bombus atratus* is an important pollinator of natural and agricultural ecosystems. Recently, the Nueva Granada University developed a rearing system of *B. atratus* colonies to evaluate its pollination activity and efficiency in traditional crops such as tomato, sweet pepper, strawberry and the native fruits, lulo and Physalis. Despite its potential as a pollinator, there was not information about effect of pesticides in *B. atratus* workers. Oral and contact toxicity tests, Lethal Dose 50 (LD50) have been used to determinate negative effects of pesticides on bees, principally in *Apis mellifera*. The present study had the goal to determine the topic and oral effect of commercial formulations of active ingredients Imidacloprid, Spinosad and Thiocyclam Hydrogenoxalate, used widely for control of different pest in Colombian crops. The LD50 were conducted in medium size *B. atratus* workers from colonies reared in laboratory,

following EPA and OECD protocols. In each test were evaluated 5 doses and controls each with 30 repetitions in replicate. Mortality was evaluated 24, 48 and 72 hours after experiment. Data were analyzed with PROBIT lineal regression model. Contact and oral LD50 for commercial formulation of a.i. Imidacloprid were 0,051 µg/bee and 0,01 µg/bee respectively. For i.a. Spinosad, contact LD50 could not be determined and oral LD50 corresponded to 0,28 µg/bee. Contact and oral LD50 for commercial formulation of a.i. Thiocyclam hydrogenoxalate were 0,244 µg/bee and 0,056 µg/bee respectively. The results contrast with those reported in other species of genus *Bombus*.

Assessing pollinator support in anthropogenic environments by applying commercial bumblebee hives: a case study around Ghent, Belgium

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Anthropogenic changes of the environment influence the distribution and abundance of pollinators such as bumblebees and have been proposed as one of the main causes in their worldwide decline. In order to evaluate the impact of expanding anthropogenic landscapes on supporting pollinator potential, reliable tools are needed. *Bombus terrestris* is one of the most abundant bumblebee species in Europe and these bumblebees are known as generalist pollinators of wild flowers in nature but also of crops in agriculture. For more than two decades these bumblebees have been commercially mass-reared for biological pollination in greenhouses.

We placed standardized commercial hives of the bumblebee *B. terrestris*, containing one queen and 40 workers, in three different locations in the region of Ghent (Belgium) and the performance of these hives was followed during a four-week period in springtime. In parallel, we determined the floral richness and diversity index in the chosen study sites. The sites consisted of a rich urban environment with patchy green areas opposed to an urban environment with poor landscape metrics; a third rural study site showed average positive landscape metrics.

The results demonstrated that the parameters “hive biomass” and “numbers of workers” increased significantly in the rich compared to the poor environment, providing a mechanism to discriminate between study sites. Finally, the results are discussed in relation to the optimization of the experimental setup and to the use of commercial bumblebee hives in assessing local pollinator support within any given environment.

Mapping European Nectar Flows using MODIS Satellite data and Honey Bee Scale Hives

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Insect pollination is vital to both ecosystem health and food production and it is important to understand how the phenology of both pollinators and plants will be influenced by global climate change. Advances in satellite technology have allowed a near-daily synoptic view of the earth but it is difficult linking the imagery with in situ events. The MODIS Aqua and Terra satellites produce the Normalized Differentiation Vegetation Index (NDVI) which is able to show the “green-up” or start of spring. Locally, the phenology of nectar-producing flowers can be seen through the honey bee nectar flow (HBNF), which is measured by the daily weight gain of a colony of honeybees. Using scale data from 2009-2012 in Slovenia, Switzerland, Belgium and Finland, this research investigates the link between satellite data and scale hives in a variety of DMEER ecological zones. Preliminary results show a strong, predictive relationship within the Broadleaf forests of central Europe, indicating it is possible to use continental MODIS climate models to predict honey flows in this ecozone.

Honeybees and bumblebees: two ways of living lead to differences in immune responses and fitness costs

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Studying the interdependences between bees’ immune responses and related fitness costs provides a comprehensive approach to assess colony constraints. Recent studies show that the variations in immune responses among different taxa of insects point to different fitness costs in relation to the complexity of such responses. Using three bacterial species, we investigated whether a prior bacterial encounter could provide protection against a later exposure to the same or a different bacterium. This phenomenon of long-lasting induced protection has been referred to as immune priming. Additionally, we assessed fitness costs associated with an activation of the immune system at the individual level in honeybees and bumblebees and at the colony level in honeybees. For this purpose, we compared the flight performance of honeybees and bumblebees and the acceptance behavior of nest-mates toward immune-challenged honeybees in regular colonies. Our results show that despite triggering the immune system of honeybees, no protective effects to reinfections were found. Furthermore, honeybees were not affected by an immune-challenge in their flight performance whereas bumblebees showed significant flight impairment. Regarding experiments at the colony level, immune-challenged honeybees showed lower survival rates than naïve individuals when introduced into a regular colony. The present investigation discloses different immune response-cost scenarios in honeybees and bumblebees

for the first time. This may help to shed light on underlying evolutionary and ecological restraints and to better understand the observed decline of these vital pollinators.

Physiology

Standard metabolism at high ambient temperatures and critical thermal maxima of *Apis mellifera carnica* and *A. m. ligustica*

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Apis mellifera carnica (“Carniolan bee”) and *A. m. ligustica* (“Italian bee”) are closely related subspecies of the Western honeybee, living in neighboring regions. The Carniolan bee is the honeybee of the temperate Central European climate region, ranging from the Alps to the Carpaths. The Italian bee is originally native to the region of the Apennine peninsula with characteristically Mediterranean climate with its dry, hot summers and mild winters. Temperature is a major abiotic parameter which influences nearly all physiological and biochemical processes, thus determining a great deal of animal life. Standard metabolism and upper critical thermal limits are crucial physiological traits, adapted to environmental and climate conditions in the evolutionary process. We investigated whether samples from these two honeybee ecotypes differ in these traits.

The standard metabolic rate was determined from the bees’ CO₂ emission via flow through respirometry in the range of 30 to 40 °C. It was higher in the *A. m. ligustica* than in the *A. m. carnica* sample only at the highest experimental temperature ($T_a \sim 40$ °C: $dVCO_2 = 12$ nl s⁻¹; $P < 0.05$), probably due to higher body temperature ($dT_{thorax} = 1.5$ °C; $P < 0.01$). The critical thermal maxima of activity (actCT_{max}, via IR thermography video sequences) and respiration (respCT_{max}, via respirometry) were similar (difference in actCT_{max}: 0.8 °C; $P > 0.1$, respCT_{max}: 1.1 °C; $P > 0.05$). Our results reveal the adaptation of the two subspecies to their historic climate conditions, possibly favoring *A. m. ligustica* in a warming environment.

Effect Of Determination *Ferula communis* And *Pimpinella anisum* On Honey Bee Colony Performance And Brood Production

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Turkey has enormous beekeeping potential having rich flora, suitable climatic and ecological conditions. In terms of honey production, Turkey has been ranked fourth in the world, with 73 929 t produced in 2006 (ŞAHINLER et al. 2003 ;ANONYMOUS, 2006).

Honey bees require carbohydrates, proteins, fats, minerals, vitamins, and water for growth, development, maintenance and reproduction. The natural foods of the honey bees include pollen, nectar, honeydew or honey and water. Supplementary feeding enables the colony to survive and in turn ,make it more populous. Furthermore it enables the colony to

produce more honey or to pollinate crops better (HAYDAK, 1970).

Some researchers reported that supplementary feeding of the honey bee colonies with sucrose syrup and pollen substitute increases the colony performance and honey yield (JOHANSSON and JOHANSSON, (1977); KUMOVA et.al. (1993); AKYOL (1998); GÜLER (2000); KUMOVA (2000); ŞAHINLER and KAYA,(2001)).

In this study, Muğla (*Apis mellifera anatoliaca*) honey bee colonies were used to investigate the effect of *Ferula communis* and *Pimpinella anisum* on colony performance and brood production. The study was carried out in Hatay and Konya provinces. The experimental colonies were fed by sucrose syrup and pollen substitute (bee cake) with *Ferula communis* and *Pimpinella anisum*.

The research findings point out that 45 % percent increased in brood production in group which were fed with pollen substitute include 25 % *Ferula communis* extract autumn season in Hatay compare to control group. In addition, they point that 25 % percent increase in brood production in group which were fed with in pollen substitute include 25 % *Ferula communis* extract compare to control group in Konya.

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Hydroxymethylfurfural (HMF) in sugar candies affects lifespan of honeybees in hoarding cages

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We found increased hydroxymethylfurfural (HMF) concentrations in sugar candies that were purchased on the market in Slovenia in the spring of 2013. High levels of HMF could potentially have negative effects on bee longevity. The aim of the study was to test the survival of winter bees fed by candies with various concentrations of HMF. Winter bees from colonies were collected and inserted in hoarding cages. Caged bees in each of five treatment groups were fed with: (A) 'MedoPip Standard' (914.6 mg HMF/kg), (B) 'MedoPip Plus' (437.0 mg HMF/kg), (C) 'Apimel' (58.3 mg HMF/kg), (D) 'home-made' sugar candy with 10 % water and (E) 'Stimulans' candy (<10.0 mg HMF/kg). Populated cages were kept in an incubator, candies and water were offered separately and prepared freshly each day and dead bees were also counted daily. Bees fed with 'MedoPip Standard' or 'MedoPip Plus' candies significantly induced ($p < 0.0001$) faster bee mortality in comparison to bees fed with other diets. The longest survival of winter bees was found in those fed 'Apimel', 'home-made' or 'Stimulans' candies, where bees survived in average of 27 days. 'Home-made' or 'Stimulans' candy had the lowest HMF concentration (detected by HPLC) of all tested candies. However, the bees fed 'Medopip Standard' and 'Medopip Plus' candy had a shorter life span (24 and 20 days, respectively). Our preliminary results indicate the potential adverse effect of high concentration levels of HMF, contained within the purchased sugar candies, on the honeybee lifespan.

Social temperature homeostasis in a breeding honeybee colony

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Social colony temperature homeostasis allows honeybees to promote a constant speed of brood development even in the variable environment of temperate climatic zones. Beside endothermy on demand bee density is an important parameter of colony homeostasis. We investigated the interaction of thermoregulatory and behavioral measures for brood temperature stabilisation in a broad range of environmental temperatures (T_{env} = 13–41°C). Bees (*Apis mellifera carnica*) were kept in an 8-comb observation hive. Opening of the hive between the two central combs gave fast access to the interior for thermographic snapshots. We measured the location and surface temperature of all bees visible on the central comb, and the comb surface temperature. Air temperature near the bees (T_a) was interpolated from a mesh of 26 thermocouples. On the brood nest, the mean thorax surface temperature (T_{th}) increased from 34.3°C to 36.3°C at T_{env} = 13 and 40°C, respectively. T_{th} was lower than T_{comb} at T_{env} < 30°C, and higher at T_{env} > 30°C. T_a remained always below T_{th} and T_{comb} . Total bee density decreased with increasing T_{env} from ~285 to ~137 bees per comb at T_{env} = 13–40°C, respectively. In a similar way, the density of endothermic bees (thorax at least 0.2°C warmer than other body parts) decreased with increasing T_{env} from ~106 to ~40 bees per comb. Our experiments deliver the first quantitative approach to the degree of endothermy and regulation of bee density in a honeybee colony in reaction to environmental changes. The bees coordinate both parameters to achieve optimal brood development in a variable environment. Supported by Austrian Science Fund FWF-P20802.

The viability loss of spermatozoa in drones during eversion of endophallus

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Earlier studies showed that the viability of spermatozoa in the seminal vesicles of drones is higher than in the lateral oviducts of the queens. Accordingly, we thought that there must be viability loss during eversion of endophallus. We aimed to determine whether the process of eversion of endophallus causes the viability loss of spermatozoa in ejaculates of drones. To do this, we detected the viability of spermatozoa in semen samples obtained from intact seminal vesicles and partly and fully everted endophalli of drones, since the eversion of the endophallus during mating occurs at two steps; as partial eversion and full eversion. The results showed that the mean viability of spermatozoa in the bulb of partly everted endophallus (97.7%) did not differ significantly from that of spermatozoa in the intact seminal vesicles (98.1%). Thus, no significant spermatozoa viability loss was detected during the partial eversion of the endophallus. In contrast, the spermatozoa viability diminished significantly ($P < 0.01$) from 98.1% to 94.8% (viability loss: 3.3%), when the eversion was completed. Further, the viability of spermatozoa diminished significantly

($P < 0.01$) to 87.0%, when the semen in the bulb of the partly everted endophallus was artificially exposed to additional pressure. However, the viability of spermatozoa on fully everted endophallus (94.6%) did not diminish, when the ejaculate on the endophallus was exposed to aeration for 1 minute. We, therefore, concluded that the acting factor decreasing the viability of spermatozoa was the increased pressure in the organ necessary for full eversion.

Influence of the presence of young workers and of brood on worker longevity in honeybees *Apis mellifera*

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Honeybees (*Apis mellifera*) distribution ranges from tropical to temperate climate zones. In order to cope with important environmental variations in temperate regions, honeybees have evolved a complex life history strategy based on short living summer bees and long living winter bees. Multiple external factors and within-hive stimuli, such as colony population dynamics, influence worker longevity. Brood presence and therefore the performing of brood care has been shown to affect workers longevity. However, experiments testing this effect through brood removal also prevented the emergence of new generations of workers, which influences population dynamics and might affect individual longevity. We tested the effect of the presence of freshly emerged workers on nest-mate longevity. We show, using survival analyses, that the addition of freshly emerged bees under brood-free conditions reduces workers longevity. This effect seems to be stronger in reducing worker longevity than that of brood care. Our results bring new insights in the regulation mechanisms of worker longevity, a parameter of high plasticity, which is central to the life history of the honeybee society.

Influence of environmental factors on the behavior of the stingless bee *Melipona subnitida* in the Brazilian tropical dry forest

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Environmental conditions exert a strong effect on internal and external activities of social bee colonies. Here, we evaluated the influence of environmental factors on pollen foraging and brood cell construction of colonies of the stingless bee *Melipona subnitida* (Apidae, Meliponini), one of the few social bee species naturally occurring in the Brazilian tropical dry forest. The climate of this ecoregion in northeastern Brazil is classified as

semiarid with elevated annual temperatures and extended periods of drought. Most flowering plants are in bloom only during a very short and irregular rainy season. Our study showed that ambient temperature and the time of sunrise determined the time of foraging onset and the time of peak activity. Resource availability, on the other hand, influenced both maximum number of foragers and the end of foraging activity: the more plants were in bloom, the larger was foraging force and the later ended foraging activity. In the hottest and driest months, both foraging activity and the rate of brood cell construction were strongly reduced. Thus, our study provides the first evidence for behavioral adjustments of stingless bees to the extreme environmental conditions met in the Brazilian tropical dry forest: (1) Colonies collect large amounts of resources during a short period of the day and, also, during the year, directing the foraging force to the most lucrative sources. (2) Reduction of brood cell construction is an important mechanism for preserving the pollen supply and, thus, for increasing the chances of survival during long periods of resources scarcity.

Haemocytes of worker bees

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Analyse of the honeybee haemolymph would be used as a simple diagnostic method for estimation of healthy status the honeybee colony. We set the hypothesis that different level of condition and nutrition influences the type spectrum and/or total number of haemocytes. Samples of haemolymph were taken from adult worker bee abdomen using fine glass capillary tube. Samples can be spread onto a glass slide air dried and fixed by methanol. The Pappenheim panoptic staining was used as an initial procedure in the identification of any haemocyte type. Coatings are then viewed with an optical microscope with immersion lens at a magnification of 1000 x. We found three very abundant types of haemocytes (prohaemocytes, granulocytes and plasmodocytes) and sparsely represented the fourth type (spindle-like cells). Significant differences were found between bees from bee colony placed in flight room and outdoor situated colonies. Next differences were found depend on season (spring/late summer). This method will be applied in more colonies during the year and will combine with more biochemical characters of haemolymph to obtain a mirror of normal and not normal status of honeybee colony.

Bee Products

Anti-melanogenesis effect of water soluble royal jelly in α -MSH stimulated B16F1 cells

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Royal jelly has been widely used for health in the world. However, royal jelly sometimes cause allergy, and we develop water soluble royal jelly (WSRJ) which is removed allergen. In this study, we focused to identify anti-melanogenic efficacy of WSRJ. To access WSRJ as an effective whitening agent for cosmetics and potential external treatment for topical use, we investigated its ability to inhibit tyrosinase activity and subsequent melanin biosynthesis. B16F1 melanoma cells were treated with 10 nM α -melanocyte stimulating hormone (α -MSH) and then with various doses of WSRJ. WSRJ inhibited direct tyrosinase activity and cellular tyrosinase activity, which decreased melanin synthesis in α -MSH stimulated B16F1 melanoma cells and which was comparable to that of arbutin. In addition, we investigated mRNA and protein expression of melanogenesis-related genes such as tyrosinase, tyrosinase related protein-1 (TRP-1) and TRP-2 by reverse transcription-polymerase chain reaction and western blotting. WSRJ decreased the mRNA and protein expression of tyrosinase, TRP-1 and TRP-2, which was comparable to those of arbutin. In conclusion, WSRJ has strongly anti-melanogenic activity that is exerted by direct inhibition of tyrosinase activity and by suppression of the expression of melanogenic related genes. This study suggested that WSRJ has potential to use as a candidate for the treatment of skin pigmentation.

* The authors contributed equally to this work and should be considered as co-first authors.

Role of honey bee on the yield and yield contributing characteristics of rapeseed and mustard (*Brassica rapa*)

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The experiment was conducted in the field of Oilseed Research Centre, Bangladesh Agricultural Research Institute Joydebpur, Gazipur during rabi 2012-13. This investigation consists of the experiment i.e. impact of bee pollination on the yield and yield components of *Brassica rapa* (var. BARI Sarisha-14). In bee pollinated (BP) plants the number

of pods per plant, number of seeds per pod, weight of 1000 seeds (g) and yield/ ha were about 17.70%, 24.79% , 28.45% and 15% higher than naturally/open pollinated (NP) plants respectively. Bee pollination is most effective and cheaper device for seed production of rapeseed and mutard (*Brassica rapa*).

The antioxidant activity of German propolis – the effect of polyphenols and flavonoids

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Propolis can be divided by thin layer chromatography (TLC) into chemical profiles according to their flavonoid pattern, which depends on the specificity of the local flora. German propolis shows 2 main different types of pattern, an orange-type (O-type) and a blue-type (B-type). This study aimed to determine the correlation between the antioxidant activity, the total polyphenols and the total flavonoid content calculated by adding up the two groups of flavone/flavonols (FF) and flavanones/dihydroflavonols (FD).

Ethanol extracts of 13 propolis samples were prepared (5 B-type and 8 O-type propolis) using 2 ethanol concentrations (96% and 60%). As we knew the balsam content of our propolis samples, propolis extracts were prepared with the defined balsam contents that allowed a correct evaluation of our results. Antioxidant activity was investigated by means of free radical scavenging activity (DPPH assay), total polyphenols were determined by using Folin-Ciocalteu colorimetric method, flavone/flavonols (FF) were determined through the spectrophotometric assay based on Al (III) complex and finally, flavanones/dihydroflavonols (FD) were determined with a method based on the interaction with 2,4-dinitrophenylhydrazine (DNP) in acidic media.

The correlation between polyphenolic profile of propolis and its antioxidant activity was found to be positive, as well as between flavonoid contents and antioxidant activity. The highest antioxidant activity was found in O-type propolis extracted in ethanol 60% and the lowest activity in B-type propolis extracted in ethanol 96%. O-type propolis showed higher amounts of total polyphenols and flavonoids than B-type propolis.

Phenolic compounds in different types of Spanish honey.

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Identification and quantification of phenolic compounds in Spanish honey samples were carried out in this study. In total fifteen honey samples from different floral and geographical origins were analyzed by high performance liquid chromatography (HPLC)

coupled in series to an Esquire 1100 ion-trap mass spectrometer (IT) equipped with an electrospray interface (ESI) according to Truchado, Ferreres and Tomas-Barberan (2009) with slight modifications. Phenolic compounds were identified by UV spectrum, retention time, molecular mass and MSn fragments and when was possible through comparison with standards. Quantification was carried out with calibration curves of quercetin, chrysin, hesperetin and rutin. In all samples propolis-derived compounds (chrysin, pinobanksin, pinocembrin, quercetin or kaempferol) were found. The presence of some floral markers in some types of honey such as rosmarinic acid, kynurenic acid, myricetin or tricetin allowed us to confirm the floral origin of honey, previously studied by palynological analysis. Significant differences between the types of honey studied related to the quantity of phenolic compounds detected were found, in general dark honey samples presented more phenolic compound than light ones.

Pilar Truchado, Federico Ferreres, Francisco A. Tomas-Barberan. (2009). Liquid chromatography–tandem mass spectrometry reveals the widespread occurrence of flavonoid glycosides in honey, and their potential as floral origin markers *Journal of Chromatography A*, 1216, 7241–7248

Quality evaluation of honey produced in Veneto region from 1998 to 2013

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In 1998 our laboratory started the evaluation of the quality of honey produced in Veneto region (Northern Italy), in collaboration with regional beekeepers associations, that provide every year a representative number of newly produced honeys, with a total of 1,059 samples. Honey quality is evaluated by the quantification of physico-chemical parameters (i.e. moisture, HMF, diastase, etc.) and environmental and pharmacological residues. Moisture, HMF and diastase are considered markers of the freshness of honey and the overall pattern of the experimental results shows that these parameters are in compliance with existing regulation, Council Directive 2001/110/EC. The concentration of two representative heavy metals, cadmium and lead, have been investigated as markers of environmental pollution. Cadmium was always found at low concentration, and with a decremental trend (from 0.014 to 0.001 mg/kg). As expected, lead concentration decreases in time (from 0.176 to 0.060 mg/kg), correlating with the increased world-wide use of green fuel. Among the contaminants from beekeeping the honey samples have been tested for acaricides and antibiotics and chemotherapies (sulphonamides, tetracyclines, streptomycin, tylosin). Only 5 samples were found positive for acaricides (coumaphos and/or fluvalinate) and 6 for sulphonamides (4 for sulfathiazole, 1 for sulfamethazine, 1 for sulfadiazine), with the last positive sample detected in 2006. The experimental data show a stable high quality of honey produced in Veneto region, concerning the physico-chemical parameters, with a tendency to improve due to the decreasing in concentration of cadmium

and lead, and the lack of pharmacological residues since 2006.

Determination of resveratrol and piceid isomers in beeswax

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In the last decade, a high mortality of honeybees (*Apis mellifera*) in different areas of Spain, and in many other countries around the world, has been detected. The factors that influence this honeybee mortality are numerous and they have a high degree of interaction between them, which make difficult their identification. One of the possible causes of the honey bee mortality is related with massive presence of *Nosema ceranae*, which is a microsporidium found worldwide and it is considered to be one of the main honey bee pathogens. One of the compounds that was effective against nosemosis (*Nosema Apis*), fumagillin, has also shown to be effective against *N. ceranae* in controlled experiments, but its use is not currently authorized in the EU. Thus, it is essential to develop new molecules that could be successfully used instead of fumagillin. Nowadays, the potential of natural compounds like phytoalexins, and in particular resveratrol and its related compounds (isomers and piceid), for controlling *Nosema* infection in honeybees is being investigated. It will therefore be necessary to develop analytical methodologies to assess their potential presence in bee products, like beeswax, due to the fact that beeswax could be considered a reservoir for the beehive, and the products present in beeswax could directly affect the bee colony or be transmitted to other bee products. In this study, a new liquid chromatography method coupled to electrospray mass spectrometry has been developed for the determination of residues of resveratrol in all its forms in beeswax.

Acknowledgements

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Radical Scavenging Activity of Ultrasonic solvent Extracts from Dalmatian Sage Honey Samples

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Honey aroma, one of the most typical features of honey, is very complex and involves many tens of volatiles compounds. One of the common honeys from Dalmation region of Croatia is Sage honey. Isolation of volatiles from two samples of *Salvia officinalis* L. uni-

floral honey using one methods ultrasonic solvent extraction (USE), followed by gas chromatography and mass spectrometry analyses (GC, GC-MS). The solvent used for USE was mixture of pentane and diethyl eter (1:2 v/v). Benzoic acid and phenylacetic acid where the major compounds of ultrasonic solvent extracts. The scavenging ability of the series of concentrations of the honey ultrasonic solvent extracts and the corresponding honey samples was tested by DPPH (1,1-diphenyl-2-picrylhydrazyl) assay.

Nectar production and attractiveness of selected apple cultivars (*Malus domestica* Borkh) to honeybees

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Attractiveness of four apple cultivars (*Golden Delicious Reinders*, *Gala*, *Granny Smith* and *Idared*) related to nectar foraging preference of honeybees (*Apis mellifera* L.) was investigated. The study included determination and comparative analysis of the total daily nectar amount per flower, diurnal dynamics of nectar secretion, nectar secretion rate as well as the frequency of honey bee visits, in order to determine the most attractive source of nectar during the domination of this bee pasture.

The intensity of nectar secretion was determined by the microcapillary method. Nectar sampling, observation of honeybee visits and measurement of microclimate parameters (air temperature and humidity) were carried out in three-hour intervals during the daytime.

The maximum total daily nectar amount per flower (average 1.483 µl) and the rate of nectar secretion was found in *Idared* cultivar (0.124 µl/h). Examining the diurnal dynamics of nectar secretion (7 am to 7 pm), it was found that all cultivars secreted nectar continuously during daylight, whereas nectar amounts per flower irregularly fluctuate. Bees visited flowers of all cultivars continuously from morning till evening, either with almost equal frequency (*Golden Delicious Reinders*) or the visits were slightly increased in the morning followed by decreasing tendency towards evening (*Gala*, *Granny Smith* and *Idared*). Honeybees most frequently visited the *Idared* flowers (5 bees in 5 minutes).

Among the analyzed apple cultivars, *Idared* and *Granny Smith* represent more important source of nectar, essential for the successful development of honeybee colonies in the spring. Therefore, more intense cultivation of these cultivars is recommended to increase honey yield.

Antitumor activity of methanol pollen extract on murine colon cancer cell line

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In order to evaluate the antitumor activity of 80% methanol beepollen extract, one sample with identified botanical origin, chemical composition and biological activity has been used on murine colon carcinoma C26 cells. The floral origin of bee collected pollen identified by palynological analysis was: predominant pollen (>45%) *Filipendula ulmaria*, secondary pollen (16-45%) *Calluna* sp., important minor pollen (3-15%) *Artemisia* sp. and *Hypericum* sp. and minor pollen (<3%) *Epilobium* sp., *Taraxacum officinale* and *Geranium* sp. Total polyphenols content determined by the Folin-Ciocalteu colorimetric method was 6.33 ± 0.16 mg GAE/g dry beepollen and total flavonoids content determined with zirconium oxychloride reagent was 4.52 ± 0.13 mg QE/g dry beepollen. The antioxidant properties evaluated by 2,2-diphenyl-picrylhydrazyl radical scavenging capacity (DPPH) assay was 1.14 ± 0.02 mmolTROLOX/g dry beepollen, while the ferric ion reducing antioxidant power (FRAP) assay indicated a value of 0.60 ± 0.04 mmol Fe^{2+} /g dry beepollen. Antitumor activity on C26 cell line has been assessed by cell proliferation (MTT) and apoptosis (APOPercentage Apoptosis Assay kit). Cell cultures were treated for 24 h with 0.25, 0.5 and 1 mg/ml 80% methanol beepollen extract. The antiproliferative activity (between 26.03 ± 4.59 and $50.29 \pm 9.23\%$) was correlated with apoptotic index (between 4.33 ± 0.26 and $27.30 \pm 4.64\%$). Results showed that methanol beepollen extract inhibited cell proliferation and induced apoptosis in murine colon cancer cells. Our findings suggest that the effects of polyphenols and flavonoids on colon cancer cells were observed in a dose-dependent manner. This effect remains to be further explored.

Protein content in European bee pollen balls monospecific

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Bee pollen (BP) is an important natural product used therapeutically in medicine, pharmaceutical industries and as nourishment. Content of macro- and micronutrients of BP, and the influence of the botanical origin, has been published in many papers (Vil-lanueva et al., 2002, among others). Variabilities in protein content seems to depend more on plant diversity rather than the method of preservation. Usually, protein content is measured in the total sample. Few studies exist on the protein content in monospecific BP balls. Only Szczesna (2006), found that Brassicaceae family and *Chelidonium majus*, was characterized by a high protein content (24.54%), and *Artemisia* and *Polygonum bistorta* by a low protein content (13.06%). We have analysed bee pollen samples from Iberian Peninsula, Turkey, Italy, Bulgaria, Greece, Finland and France. The aim of this study is to know:

a) the protein content in monospecific European bee pollen balls; and b) which botanical species, or families, of European flora provide the highest protein content to BP. Kjeldahl method, in dry BP at 40 °C, was applied to determined protein content. Our results showed that, in European BP protein content mean values range between 17–24%, being very variable between samples and countries. Cistaceae is the family which has a lower protein content (13–19%), followed by Compositae (15–21.53%), Brassicaceae (18.61–24%), Ericaceae (18–24.42%), Quercus (18.59–24.55%), Liliaceae (19.57–20.44%), and Castanea (21–22.75%). On the other hand, families with higher protein content are Labiatae (23.67–33.61%), Leguminosae (18.65– 31.68%) and Rosaceae (18.20–29.13%).

Comparison of phenolic compounds and antioxidant activity of hawthorn (*Crataegus monogyna*) honey, herbhoney and plant extracts

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Evaluation of honey quality, authenticity and lately its therapeutic properties, are the main targets in beekeeping research. Hawthorn is one of the most valuable and effective herbs for the heart, being used for the treatment of cardiac diseases since ages. Flowers and leaves contain different phenolic acids and flavonoids (both aglicons and glycosides). The present study aimed to characterize alcoholic and water extracts from *Crataegus monogyna* (hawthorn) leaves and flowers and comparing with hawthorn honey chemical composition. Water and ethanolic extracts (5%) were prepared from dry powdered hawthorn flowers and leaves and subjected to chromatographic analysis of polyphenolic profile. Same protocol was used for organic hawthorn honey and hawthorn herbhoney (a mixture of honey and fresh hawthorn leaves and flowers). Different quantities of caffeic and chlorogenic acids were quantified in hawthorn extracts and herbhoney. Flavonoidic aglicones and glycosides such as rutin, apigenin, quercetin, luteolin, luteolin-3,7-glucoside and apigenin-7-O-glucoside were identified in honey and extracts. High antioxidant properties (radical scavenging activity, reducing power and inhibition of lipid peroxidation), were observed in hawthorn extracts and herbhoney. The potential of *Crataegus monogyna* plant, honey and herbhoney as important source of biologically active compounds and nutraceuticals was demonstrated by the present study.

The importance of some sunflower hybrids for apiculture in Hungary

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Sunflower is the most important oil crop in Hungary. Its sowing area has varied between 550-600 thousand hectares in the last couple of years. From the beginning of the eighties sunflower has been increasing its importance as a summer bee pasture, available almost all parts of the country. Migratory beekeepers meet mostly with oil-type sunflower hybrids although their nectar production and bee attraction (visiting) can be very different.

The aim of the study in 2013 was to determine the bee visitation and apicultural value (nectar and pollen production, sugar concentration of nectar) of 13 different sunflower hybrids. The influence of the main climatic factors on the quantity of secreted nectar and on sugar concentration of the nectar were also determined.

The results show that environmental factors such as fast wind and high temperature can diminish insect activity, nectar production day by day. It was also demonstrated that nectar production is highly influenced by agro-ecological conditions and genetic background of the hybrid.

Significant differences were observed in the apicultural value (nectar production, sugar concentration of nectar and sugar value) and in insect activity of various sunflower hybrids. The activities of the insects began at early morning (around 7 am) and closed 17 pm.

Microbiological evaluation of dehydrated bee-pollen produced in Brazil

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Bee-pollen is an apicultural product with potential for medical and nutritional applications; therefore, its microbiology quality should be monitored. The objective of this study was to enumerate aerobic mesophilic and psychotrophic bacteria, yeast and molds and to determine the presence or absence of sulphite-reducing *Clostridium* spores and *Salmonella* of eight dehydrated bee-pollen collected from April to July of 2012, in Brazil. Each sample, weighing 10 grams, was homogenised and serial dilutions were inoculated on specific medium. Afterwards, incubations were performed depending on the microorganism. Results were expressed as colony-forming units per gram of dehydrated bee-pollen (CFU.g⁻¹) for mesophilic, psychotrophs and yeasts and molds or as presence or absence for

sulphite-reducing *Clostridium* spores and *Salmonella*. Total aerobic mesophilic counts ranged from $6.8 \times 10^2 \pm 8.8 \times 10$ to $3.0 \times 10^3 \pm 4.8 \times 10^2$ CFU.g⁻¹; psicotrophic counts ranged from $<1.0 \times 10$ to $3.5 \times 10^2 \pm 7.5 \times 10$ CFU.g⁻¹; while the values for yeasts and molds were between $1.6 \times 10 \pm 0.5 \times 10$ and $2.0 \times 10^3 \pm 4.1 \times 10^2$ CFU.g⁻¹. All the samples were absent regarding sulphite-reducing *Clostridium* spores and *Salmonella*. Microbial contamination of this product can occur through primary sources such flower pollen, nectar, environment and bees or secondary sources, during collection and processing by beekeepers. According to the literature available, the bacterial and yeasts and molds counts identified were usual for that product, but it is important to consider that these microorganisms can cause spoilage and diminish shelf life, so quality control programs should be implemented. Lastly, the absent of sulphite-reducing *Clostridium* spores and *Salmonella* was important to ensure the consumer health.

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Proteom based comparison of venom extraction methods for *Bombus* sp.

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The aim of the study is to compare the protein profiles of *Bombus* (*Bombus*) *terrestris* venom by using two different extraction methods; disruption of venom sac and manual milking. Protein concentrations were determined from each group, followed by two-dimensional gel electrophoresis and peptide mass fingerprinting analysis by using matrix-assisted laser desorption ionization/ time of flight mass spectrometry.

A total of 111 protein spots were observed on each gel in PDQuest analysis. These spots were then subjected to student's t-test both in PDQuest and SPSS. According to our results, there were no statistically significant difference in protein spots between the two extraction groups. The proteins that are identified from *B. terrestris* venom obtained by using two different methods are; Pseudohemocyanin-1 (Fragment), L-2-hydroxyglutamate dehydrogenase mitochondrial, actin-2 muscle-specific, arginine kinase, S-methyl-5'-thioadenosine phosphorylase, phospholipase D LvSicTox-alpha1C1aiii (Fragment), Serine/threonine-protein phosphatase PP1-beta, venom protease, venom allergen 5,02 (fragment) and potassium channel blocker alpha-KTx 26.1.

Results of the present study suggest that protein profiles of venom samples obtained from disruption of venom sac and manual milking are similar. Importantly, the results show the potential of disrupting the venom sac and that it can also be favoured as a venom extraction method for bumble bees.

Effects of Giant Honeybee (*Apis dorsata* Fabricius, 1793) Venom on Renal Failure in Wistar Rat (*Rattus norvegicus* Berkenhout, 1769)

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The effects of giant honeybees (*Apis dorsata*) venom on renal failure in male rats (*Rattus norvegicus*) were investigated. Three dosages of venom were prepared by dissolving in 0.85% NaCl (normal saline); 30.0 µg/g bodyweight (high dose, HD), 20.0 µg/g bodyweight (low dose, LD) and 0.0 µg/g bodyweight for the control (CO). Two-hundred µl of each dose was injected in rats. After 24 hours, the renal tissues were collected and processed by paraffin technique, stained with periodic acid-Schiff's reagent (PAS) and Masson's trichrome stain for histological structure analyses. The findings show that Bowman's space of HD rats were significantly more narrow than those of LD and CO ($F_2=3.94$, $p\leq 0.0201$). Moreover, the percentage of destructural glomeruli of HD was significantly higher than those of LD and CO, which were $44.14\pm 2.41\%$, $29.65\pm 1.20\%$ and $10.76\pm 1.74\%$, respectively ($F_2=676.02$, $p\leq 0.0001$). In addition the HD rats also showed the highest injury of brush borders of proximal convoluted tubules ($F_2=848.09$, $p\leq 0.0001$). The impaired tubular cell nuclei were found in the dosed groups. These results indicate that the experimental dose of bee venom can cause signs of renal failure.

A new and bee friendly application technology in oilseed rape

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To prevent losses of yield, oilseed rape has to be treated with different pesticides during blooming season. The application of pesticides into the blossoms has led to residue findings in honey which caused concerns by beekeepers. We present a concept to optimize pesticide application technology, towards minimization of exposure of bees to pesticide residues in treated blossoms.

The under-leaf application technology is a modified system (Dropleg) that has been used in vegetable crops for several years. In semi-field and field trials in 2011-13 it was tested in how far under-leaf spray with the Dropleg system in flowering oilseed rape can reduce pesticide residues in nectar and pollen collected by exposed bee colonies in comparison to conventional application technology. Pollen loads, transported nectar and stored fresh honey of experimental bee colonies were sampled at different points of time after application and analyzed for pesticide residues. As model substances, a systemic fungicide and a non-systemic insecticide were applied, in 2012 and 2013 additionally a systemic insecticide. Results from 2011 suggest that Dropleg application can significantly reduce pesticide exposure of bee colonies. Interesting advantages were found related to the drift reduction of the spray solution. The project is conducted in the scope of the FIT BEE Project supported by German BMELV and will be continued until 2014.

Advances in Chemical Ecology of Bees

Behavioural observations and analysis of cuticular hydrocarbon profile suggest chemical transparency of *Sphecodes* cuckoo bees

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Brood parasites of bees need to overcome host detection in order to invade host nests and exploit the targeted resources. Thus, brood parasites can evolve either chemical insignificance or chemical mimicry to enter the host nest without leaving traces for detection. *Sphecodes* cuckoo bees (Halictidae) are common cleptoparasites of other bee species. We made a first attempt to understand whether *Sphecodes* females use either chemical insignificance or chemical mimicry to evade host aggression, using a system composed of *Sphecodes monilicornis* and *Lasioglossum malachurum* (Halictidae). *S. monilicornis* females frequently visit the underground host nests and subsequently kill the host workers without apparent defence by the host. This is shown in circle-tube arenas, where *L. malachurum* workers rarely attack live cuckoo bees or filter-papers treated with cuticular extracts of *S. monilicornis*. Thus, *L. malachurum* seems to be weakly able to recognize the cuckoo bees as a threat, particularly in absence of visual cues. The chemical analysis of the cuticular hydrocarbon (CHC) profiles revealed that *S. monilicornis* females do not mimic the CHC profile of *L. malachurum* workers. On the other hand, *S. monilicornis* exhibits only a part of the cuticular compounds found on the host, and they do not have any additional compound. This strategy might be an alternative strategy to chemical mimicry referring to as chemical transparency. *Sphecodes* cleptoparasites may thus limit host bee overaggression by having no recognition cues.

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. In the present study, we determined

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 ($T = 25^{\circ}C \pm 2^{\circ}C$ 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.

Resistance of developing *Apis mellifera scutellata* larvae to dietary nicotine

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Honeybee larvae are often chronically exposed to pesticide residues in the food stores and wax of hives; effects of which are even less understood than for adult bees. We reared *Apis mellifera scutellata* larvae in vitro, exposing them chronically to a plant alkaloid, nicotine, to investigate how body composition, growth and survival were affected. Nicotine concentrations varied from 0 to 1000 $\mu\text{g}/100\text{ g}$ diet. Mortality was low, on average 8.4% of larvae died at the prepupal stage compared to 18.1% dying at the white eyed pupal stage. Nicotine significantly reduced survival but mass as well as body composition (protein or lipid stores) of white-eyed pupae was unaffected, with the exception of water composition. Detoxification mechanisms seem to be present in developing honeybees, enabling the larvae and pupae to resist both natural and synthetic xenobiotics, thereby explaining the lack of negative dietary nicotine effects.

Genomics

Draft genome sequence of the *Apis mellifera* L. filamentous virus (*AmFV*)

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The *Apis mellifera* Filamentous virus (*AmFV*) is a large DNA virus which sporadically produces acute infections in honey bee worker hemolymph. The virion particle consists in a long nucleocapsid folded in several loops and is visible under light microscopy. In an attempt to sequence the viral genome, *AmFV* virions were purified from infected samples through gradient centrifugation. A DNA library was produced for high-throughput DNA sequencing using Illumina technology. The assembly of the genome revealed open reading frames displaying homologies with baculoviruses. Here we present the genomic features of *AmFV* together with a draft annotated genome sequence.

A method to identify mixed pollen samples through next-generation sequencing and hierarchical taxonomic classification

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In honey bee research, the palynological identification of pollen plays an important role for the analysis of honey. Today, expert knowledge and reference collections are essential to identify pollen origin through light microscopy. Pollen determination by molecular sequencing and DNA barcoding has been proposed as an alternative approach, but so far the assessment of mixed pollen samples originating from multiple plant species is still a laborious and error-prone task requiring manual separation of pollens to taxa, each to be amplified and sequenced individually. Here we present a novel method for direct pollen assessments in mixed samples through next-generation sequencing (NGS). To demonstrate the functioning of our approach, we compared results from classical pollen determination based on light microscopy with sequencing results. The pollen samples were collected in twelve different landscapes in the region around Bayreuth, Germany. The NGS based technique resulted in higher taxon richness (deeper assignments and more identified taxa) compared to light microscopy. Abundance estimations from sequencing data were significantly correlated with counted abundances through light microscopy. Our approach presents a useful and efficient molecular and bioinformatic workflow to identify pollen at genus and species level without requiring specialized palynological expert knowledge.

Our results show that NGS based pollen determination is a useful tool to analyse mixed pollen samples from honey and field experiments at a high-throughput level.

Detection of epigenetically modified cytosines in the honey bee genome

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Epigenetic alterations, for example methylation and demethylation of cytosines, can change adult phenotypes. Forager and nurse bees show very different social feeding behaviors and also differences in fat and protein storage. How epigenetic changes can contribute to such differences is not clear.

We asked if cytosine modifications that are typical demethylation intermediates in mammals are also present in the honey bee genome.

Using liquid chromatography/tandem mass spectrometry (LC/MS/MS) we analyzed cytosine modifications in brain and abdomen of adult honey bee workers – nurses and foragers. We detected cytosine modifications, which suggest that active demethylation in the honey bee resembles similar mechanisms in vertebrates, and may contribute to worker differentiation also in bees. However, the relative amounts of different oxidized forms of methylated cytosines varied from what was expected from other systems. This indicates that the enzyme responsible for this catalysis might differ in its mode of action from its mammalian homologues.

Comparative analysis of different methods for gDNA extraction and detection of *Nosema ceranae*, *Nosema apis* and *Nosema bombi*.

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Microsporidia are highly prevalent pathogens for honey bees and bumble bees that have been related to pollinator losses. Given the high amount of clinical samples to be analyzed it is necessary to standardize a reliable, consistent and efficient technique for DNA extraction and pathogen detection. We aim in this work to compare the efficacy of three methods to extract gDNA for the detection of these pathogens: i) Biosprint (Qiagen); ii) TE buffer; and iii) a Chelex method.

To determine the limit of detection (LOD) for each method, one reference sample with purified *Nosema* spores was prepared by counting spores under a phase-contrast microscope. They were counted twice by three different people and the average was used as

a reference value. Samples were shaken in a Tissue Lyser machine (Qiagen) and processed following the three different DNA extraction protocols. After this, the DNA reference sample for each *Nosema* species was diluted into decimal series up to 1/108 and analyzed by PCR. To determine the usefulness on clinical samples, we analyzed 62 composite samples collected from honey bee colonies and 20 bumble bees (*Bombus* spp.) individually dissected to get the gut. All samples were analyzed for *N. apis* and *N. ceranae* detection and bumble bee samples were also analyzed for *N. bombi* by PCR.

All the methods were feasible for the detection of *Nosema* species in the clinical samples of honey bees and bumble bees. The lower LOD (around three times lower than the other tested methods) for detecting both *N. apis* and *N. ceranae* was obtained with the automatic method. However, the Chelex and TE methods have demonstrated to be a very good option since both are easy to use and cheaper.

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Social evolution in the sweat bees: insights from the transcriptome of the facultative eusocial *Halictus rubicundus*

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Two of the four independent origins of eusociality in the bees have occurred relatively recently (ca. 25 million years ago) in the family Halictidae (sweat bees), and several of its eusocial species are facultatively eusocial, exhibiting sociality in warmer environments with longer growing seasons and solitary behaviour in cooler environments. For one such species, *Halictus rubicundus*, mitochondrial DNA sequence data reveal the eusocial and solitary forms in the USA to be genetically differentiated, suggesting genetic underpinning to differences in social behaviour. We have generated a transcriptome of *H. rubicundus* using nextgen technologies (454 and Illumina data) and used it to map SNP variation between two eusocial populations and one solitary population in the USA. We describe our hybrid transcriptome assembly, gene prediction, annotation and SNP discovery, and we compare *H. rubicundus* with *Lasioglossum albipes*, whose genome has recently been sequenced. Though our analyses are compromised by small sample sizes, our data provide strong support for the marked genetic differentiation between the two social forms of *H. rubicundus* in the USA.

Neural basis of behavior in the honeybee

How the clock develops: The PDF-network in honeybee brains of different developmental stages

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The seasonal and daily changes in floral resources are a great challenge for honeybees. To predict the regular oscillations in the environment honeybees have evolved a circadian clock. This clock is involved in timing daily activities, in the bees' time memory and in their sun-compass orientation, and it may also be important for measuring day-length.

The molecular basis for the honeybee's circadian rhythm is a transcriptional/translational negative feedback loop in which four putative honeybee clock genes *period*, *cryptochrome-m*, *cycle* and *clock* are involved. But other factors like the neuropeptide PDF (Pigment Dispersing Factor), which has been shown to be part of the circadian clock in different insects, seem to be important for the normal circadian timekeeping, too.

Here we characterize the development of the PDF-network of *Apis mellifera* on an anatomical level via whole mount immunostainings. PDF-positive neurons of the honeybee master clock were identified in brains at different larval and pupal stages, as well as in adults. The larval and pupal stages were reared *in vitro* to achieve a higher standardization. In the very early development no PDF-positive neurons could be detected, but from larval stage 3 onward we identified PDF-neurons in the lateral brain between optical lobe and protocerebrum. The number of PDF-neurons increased throughout development and the arborization-network gained complexity. In the adult bee PDF-fibers have rich arborizations in the protocerebrum and additionally send fibers to the optic lobes, antennal lobes and ocelli. We reconstructed the arborization-pattern with ImageJ neuron tracking software and created a 3D Image.

Challenges of the risk assessment of pesticides on bees under Regulation 1107/2009.

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Regulation 1107/2009 concerning the placing of plant protection products on the market and repealing Council Directive 91/414/EEC, introduces new data requirements to assess the potential risk of plant protection products for human, consumer and the environment. These new data requirements are adopted in regulations 283/2013 and 284/2013 for

active substances and plant protection products, respectively.

Where bees (honeybees, bumble bees and solitary bees) are likely to be exposed, testing by both acute (oral and contact) and chronic toxicity, including sub lethal effects shall be conducted. Risk assessment should consider residues of the active substance in nectar, pollen and water including guttation, and the exposure from drift of dust during drilling of treated seeds, granules and slug pellets. These data requirements entered into force in January 2014 for dossiers of new active substances and for those substances whose approval expire on January 2016. In 2013, the European Food Safety Authority (EFSA), published a new guidance document to conduct risk assessment of pesticides on bees under regulation 1107/2009. The new data requirements on bees and the risk assessment schemes proposed by EFSA are currently on debate because knowledge gaps.

In this work we introduce the challenges for the implementation of new data requirements for bees under regulation 1107/2009 and the need of a holistic approach integrating multiple stressors, where the agro environmental and management conditions of the each regulatory zone defined by regulation 1107/2009 will play a significant role.

The effects of sub-lethal doses of Neonicotinoids on motor coordination of honey bees

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Our goal was to investigate the effect of sub-lethal doses of pesticides on the motor coordination of captive bees on Anatolian honey bees (*Apis mellifera anatoliaca*). We fed bees and tested the sub-lethal doses of Imidacloprid and Thiomethoxam ranging from 1/5 to 1/500 LD50 in 50% sucrose. Control bees were fed 50% sucrose only and incubated at room temperature (-control) or subjected to thermal stress (42 °C + control). Honey bees were scored for their motor coordination by assessing the proboscis extension reflex as well as coordination of the antennae, legs, and abdomen at 4 h post-ingestion. Bees that were fed doses of Imidacloprid higher than 1/100 LD50 showed reduced motor coordination similar to that of thermally stressed honey bees. Motor coordination of bees treated with 1/5 LD50 was lower than motor coordination of control bees. These results showed that Imidacloprid, even at doses 1/100th of the LD50, impaired basic motor coordination fundamental to locomotion. Sub-lethal doses of imidacloprid at 1/5 LD50 impaired the sucrose sensitivity response in honey bees. At lower doses of Imidacloprid (<1/5 LD50), bees did not perform differently than controls. Thiamethoxam also showed significant sub-lethal effects of impairing basic motor coordination of honey bees at doses substantially lower than the LD50. A single dose of Thiamethoxam at (20%) 1/5 of the LD50 impaired motor coordination of fundamental motor segments essential for foraging, including the PER, wings, legs, and abdomen, to a greater extent than heat stress. In conclusion, sub-lethal doses of both Imidocloprid and Thiomethoxam affects motor coordination of honeybees.

Appetitive and aversive olfactory learning induce similar generalization rates in the honey bee

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Appetitive and aversive learning drive an animal toward or away from stimuli predicting reinforcement, respectively. The specificity of these memories may vary due to differences in cost–benefit relationships associated with appetitive and aversive contexts. As a consequence, generalization performances may differ after appetitive and aversive training. Here, we determined whether honey bees show different rates of olfactory generalization following appetitive olfactory conditioning of the proboscis extension response, or aversive olfactory conditioning of the sting extension response. In both cases, we performed differential conditioning, which improves discrimination learning between a reinforced odor (CS+) and a non-reinforced odor (CS–) and evaluated generalization to two novel odors whose similarity to the CS+ and the CS– was different. We show, given the same level of discriminatory performance, that rates of generalization are similar between the two conditioning protocols and discuss the possible causes for this phenomenon.

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Advancing Bee Health

The Effect of Muller's Ratchet and Lack of Host Specialisation During the Experimental Evolution of Opportunistic Pathogens in Honey Bees

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Serial passage experiments (SPEs) are an invaluable tool for investigating the adaptation of parasite populations to the host. The majority of SPEs yield similar results, showing increased parasite virulence in the passaged hosts and attenuation of virulence in unfamiliar hosts. *Aspergillus* spp. fungi are the causative agents of stonebrood disease in honey bees and experimental infections have revealed that certain strains can be highly virulent. In this study, two populations of *A. flavus* and *A. phoenicis* were serially transmitted through laboratory-reared honey bee larvae to examine the evolution of virulence and fitness traits. Two host treatments were used to assess how host genetic diversity affects the evolution of these traits. In the post-selection assessment experiment larvae from the same colonies as used in the serial passages and also six new colonies, were inoculated with the ancestral and derived parasite lines to determine if there was any evidence for the evolution of host specialization. The overall effect of the derived treatments following four passages was loss of pathogen virulence and fitness with a high extinction rate. The lack of adaptation by both *Aspergillus* pathogens show how opportunistic parasites may show quite different evolutionary dynamics from those shown by specialist pathogens that are more commonly studied and demonstrate how the effect of Muller's ratchet may be substantial. Naturally occurring *Aspergillus* spp. infections are therefore more likely to be acute events rather than epidemics, which may in part explain why stonebrood epidemics in honey bee colonies appear to be uncommon.

The impact of Imidacloprid and Clothianidin on honey bee immunity

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For successful beekeeping we need healthy and resilient honey bee colonies. In recent years however, extensive colony losses occurred. The main cause of colony death is assumed to be the parasitic varroa mite that not only feeds on the bees but also transmits a number of harmful diseases. For the health of the honey bees, it is crucial to have strong defence mechanisms against those parasitic infections and diseases. This defence can be weakened by environmental factors, which may leave the bees more vulnerable for parasites and pathogens. We study the impact of agricultural chemicals on honey bee health. In detail, we investigated sublethal effects of the neonicotinoids imidacloprid and clothianidin on individual immunity. We describe the effects of imidacloprid or clothianidin on hemocyte number, phagocytotic capacity and wound healing ability of individual honey bees. Our data will contribute to a better understanding of the complex and multi-causal colony losses and may help to establish indicators for the health status in order to avoid bee damage in practice.

In vitro treatments for control of the Honey bee Nosemosis.

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Nosema ceranae is one of the most prevalent pathogens worldwide. Currently there are few substances suitable for use in controlling her. In this work, toxicity to bees and microsporicide effect of different products, that can be used to control *N. ceranae* were studied. Firstly, the toxicity of these products (Fumagillin, Resveratrol Toltrazuril and Sulforaphane) was determined in *Apis mellifera iberiensis* following the recommendations of the OECD / DAC 214. For evaluate the microsporidicidal effect, we utilized newborn bees obtained in laboratory that were randomly caged (15 bees per cage, 3 replicates per dose and product). Later, following the protocols developed in our laboratory, each individual bees were infected with 100,000 fresh *N. ceranae* spores. After infection, bees were fed *ad libitum* with syrup of water and sugar (50%) during 5 days. The fifth day after infection, we added a new syrup with a dose of 7 g / L of Fumagillin, Resveratrol, Toltrazuril, Tannic acid and Sulforaphane 175ppm, and an group received only syrup, for 5 days. The evolution of infection in individual bees (five ventricles for experimental group and sampling point) was determined with a qPCR protocol at 24, 48, 72 and 96 hours after the administration of the different products. Low toxicity to bees and the significant effect to avoid the exponential growth of *Nosema ceranae* in the ventricle of bees, suggests that the studied products could be an alternative for the control of *Nosema* in field conditions.

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