ABSTRACTS

CEREBRO FAVCIBVS VTERO
AB ORBIS ORIGINE
TENENT

EURO I.U.S.S.I.
26-30 August 2012
Montecatini Terme
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Italy

I.U.S.S.I. SEZIONE ITALIANA

5th ConGREss of the EUROPEAN SECTIONS

of the International Union
for the Study of Social Insects

26/30 August 2012

MONTECATINI TERME *(Tuscany, ITALY)*

Sunday 26

Tuscany Inn Hotel / Terme Tettuccio

15.00 Registration

19.00 **Opening Ceremony** - *Terme Tettuccio*

21.30 Posters of the first session setup

Monday 27

Tuscany Inn Hotel

09.00 **PLENARY LECTURE**
Towards an integrated understanding of social behaviour
S. SUMNER

10.00 **Coffee Break**

Parallel Sessions – Oral Communications

FIRENZE room

GENES, GENOMES AND SOCIAL BEHAVIOUR

Eusociality, inclusive fitness theory and the major evolutionary transitions
A. Bourke

Divergent experimental selection for philopatry and dispersal affects the evolution of cooperative breeding in primitively eusocial ambrosia beetles
P.H.W. Biedermann • *M. Taborsky*

Kin-selected conflict and the evolution of lifespan and ageing in *Bombus terrestris*: queen longevity, fecundity and queen-worker conflict
E.J. Almond • *T. Huggins, G.A. Lockett, J.D. Parker, A.F.G. Bourke*

Kin-selected conflict and the evolution of lifespan and ageing in *Bombus terrestris*: age-associated transcription
G.A. Lockett • *J.D. Parker, E.J. Almond,T. Huggins, A.F.G. Bourke*

SIENA room

CHEMICAL ECOLOGY

10.20 Supercoloniality in the ant *Cataglyphis niger*
M. Baharal • *A. Hefetz*

10.40 Nestmate recognition in the red wood ant *Formica polyctena* (Hymenoptera: Formicidae)
L. Geisdorf, J. Heinze • ***M. Ayasse***

11.00 Nestmate recognition code: Variation in relative proportions of hydrocarbons may open social wasp colonies to aliens
M.C. Lorenzi • *E. Costanzi, J.P. Christides, A.G. Bagnères*

11.20 A multilevel and comparative approach to study nestmate recognition in a sympatric species complex of Neotropical ants
B. Yagound • *M. Crowet, C. Leroy, D. Fresneau, C. Poteaux, N. Châline*

Sociogenomics of conflict and cooperation in fire ant queens
F. Manfredini • *O.R. Grognuz, Y. Wurm, L. Keller, DeWayne, D. Shoemaker, C.M. Grozinger*

Egf receptor determines reproductive fate of worker bees
D. Cardoen • *E.M. Formesyn, U.R. Ernst, P. Verleyen, L. Schoofs, T. Wenseleers, D.C. de Graaf*

Genomic imprinting in the honey bee methylome
R.A. Drewell, E.C. Bush, E.J. Remnant, G.T. Wong, J.L. Stringham, J. Lim, F. Goudie • ***B.P. Oldroyd***

11.40 Pre-imaginal experience and nestmate recognition in the ant *Aphaenogaster senilis*
L. Signorotti • *P. Jaisson, P. d'Ettorre*

12.00 Mixed maternal, paternal and environmental effects on cuticular substances in *Cardiocondyla obscurior*
J. Oettler • *M.M. Pöhnl, J. Heinze*

12.20 Nest wax signals and their influence on worker reproduction in *Bombus terrestris*
A.M. Rottler • *M. Ayasse*

13.00 **Lunch**

Ploidy vs sex locus: which one determines gene expression in diploid males in fire ants?
M. Nipitwattanaphon • *J.Wang, Y. Wurm, K. Ross, L. Keller*

Insights from comparative genomics into the genome and phenotypic evolution of Hymenoptera and Formicidae lineages.
I. Pedroso • *M. Brown, S. Sumner*

Comparative genomics of fungus-growing ants
M. Schiøtt • *C. Li, S. Nygaard, S.G. Brady, W.T. Wcislo, J. Wang, G. Zhang, T.R. Schultz, J.J. Boomsma*

Attine ants: the animal freight trucks of their fungal symbiont. New molecular insight into a complex ant-fungus mutualism
P.W. Kooij • *J. Pullens, J.J. Boomsma, M.Schiøtt*

DNA methylation and caste differentiation in ants
G. Zhang • *R. Bonasio, S. Nygaard , M.Schiøtt, C. Li, D. Reinberg, J. Wang, S.G. Brady, W.T. Wcislo, T.R. Schultz, J.Boomsma*

Genetic compatibility affects division of labor in ants
R. Libbrecht • *L. Keller*

14.20 The effect of precocene, a JH biosynthesis inhibitor, on the establishment of reproductive dominance in bumblebees
E. Amsalem, P.E.A. Teal, C.M. Grozinger • ***A. Hefetz***

14.40 Smells Like Queen Spirit
J. Gowin • *K. Hoffmann, J. Korb*

15.00 Explosive backpacks in old termite workers
R. Hanus • *J. Šobotník, T. Bourguignon, Z. Demianová, J. Pytelková, M. Mareš P. Foltýová, J. Preisler, J. Cvačka, J. Krasulová, Y. Roisin*

15.20 Recruitment behaviour and communication in *Partamona orizabaensis*
I.C. Flaig • *I. Aguilar, S. Jarau*

15.40 Choosing an aphid partner: a matter of taste and smell
C. Detrain • *C. Fischer, G. Lognay, L.Diez, E. Haubruge, J. Prieur, F. Verheggen*

16.00 Do honeybees recognize eggs by peptides?
U.R. Ernst • *D. Cardoen, T. Wenseleers, P. Verleyen, L. Schoofs*

16.20 **Coffee Break**

Raising royalty: is emergency queen selection related to larval growth in *Apis mellifera*?
R.E. Mitchell • *S.E.F. Evison, W.O.H. Hughes*

16.40 Differential coding of floral and pheromonal odors by two olfactory subsystems in the honeybee Brain
J. Carcaud • *M. Giurfa, J.C. Sandoz*

Effects of natural mating and carbon dioxide narcosis on biogenic amine receptor gene expression in the ovaries and brain of queen honey bees, *Apis mellifera*
V. Vergoz • *J. Lim, M. Duncan, B.P. Oldroyd*

Queen pheromone blocks responsiveness to alarm pheromone in bees
E. Urlacher • *I. Tarr, J.M. Devaud, A. Mercer*

Aversive conditioning with heat punishment in the honeybee (*Apis mellifera*): behavior and genetics
P. Junca • *J. Carcaud, S. Moulin, L. Garnery, J.C. Sandoz*

17.00 Differences in the protein pattern of male accessory glands between winged and wingless males of two *Cardiocondyla* species
M. Fuessl • *J. Heinze, A. Schrempf*

17.20 Chemical structure of odorants and perceptual similarity in ants
N. Bos • *P. d'Ettorre, F.J. Guerrieri*

17.40 Biochemical crowdsourcing through oral fluid-exchange in ant colonies
A.C. LeBoeuf • *R. Benton, L. Keller*

18.00 ***FIRST POSTER SESSION***

with Aperitif

Tuesday 28

Tuscany Inn Hotel

09.00 PLENARY LECTURE
Collective disease defence in ant societies
S. CREMER

10.00 **Coffee Break**

Parallel Sessions – Oral Communications

FIRENZE room

SOCIAL BEHAVIOUR AND COLLECTIVE PROCESSES IN SOCIAL INSECTS

Social and spatial organization in an ant colony
D. Mersch • *A. Crespi, L. Keller*

A field study and computer simulation demonstrate how colony size and food distribution affect ant foraging rates
M.E. Moses • *T.P. Flanagan, K. Letendre*

Selection on colony personalities
A.P. Modlmeier,T. Pamminger • ***S. Foitzik***

SIENA room

DISEASES, IMMUNITY, SYMBIONTS AND SOCIAL PARASITES

10.20 Molecular evolution of immune genes in socially diverse bees
L. Viljakainen • *B.J. Fischman, S.H.Woodard, G.E. Robinson, A.G. Clark*

10.40 Diverse pathogen adaptation to individual versus social immunity in ants
M. Stock • *A.V. Grasse, S. Cremer*

11.00 Social immunisation in the invasive garden ant
M. Konrad • *M.L. Vyleta, S. Cremer*

Caste-biased gene expression underlying division of labour and dominance in a primitively eusocial ant: the role of differential gene expression
C. Asher • *P. Ferreira, H. Himmelbauer, R. Guigo, F. Nascimento, W. Hughes, S. Sumner*

Division of labor in *Cataglyphis cursor* ants determines who performs precision rescue behavior
E. Nowbahari • *J.L. Durand, K.L. Hollis*

Reduction of potential fecundity leads to premature foraging in ants – an ancestral trait in the evolution of eusociality?
B. Walter

Low relatedness can favour the evolution of eusociality
F. Muratori • *T. Wenseleers*

11.20 High socio-spatial compartmentalization supports the organizational immunity hypothesis in honeybees
D. Baracchi • *A. Cini*

11.40 Alteration of social behavioural networks in ant colonies following pathogen exposure
L.V. Ugelvig • *F.J. Theis, C. Marr, S. Cremer*

12.00 Young ant queens are attracted to fungal entomopathogens when choosing a nest site
T. Brütsch • *M. Chapuisat*

12.20 Do ants commit the Concorde Fallacy?
C.D. Pull • *M.J.F. Brown*

13.00 **Lunch**

Cannibalize this: a guide to selfishness for ant larvae
E. Schultner • *P. d'Ettorre, H. Helanterä*

Unexpected fusion of genetically divergent colonies in the invasive pharaoh ant
L. Pontieri • *Jes Søe Pedersen*

Colony fusion and reproductive conflicts in *Temnothorax* ants
N. Stroeymeyt • *J. Heinze L. Keller*

Alien queen acceptance in queen-rich and queenless colonies of ant *Myrmica rubra*
J. Sorvari

There's a right time for everything: synchronization of reproduction through policing in a clonal ant
S. Teseo • *D.J.C. Kronauer, P. Jaisson, N. Châline*

When policing fails: reproductive conflict in *Apis cerana*
M.J. Holmes • *B.P. Oldroyd, K. Tan, Z. Wang, M. Beekman*

Assured fitness returns and nest-drifting behaviour in tropical paper wasps
T. Lengronne • *S. Patalano, S. Dreier, L. Keller, S. Sumner*

14.20 Who performs undertaking behaviour in the ant *Cataglyphis velox*?
C. Westhus • *C.A. Ortega Trujillo, S. Cremer, C. Doums*

14.40 The impact and dynamics of a multi-host nematode parasite of bumblebee queens
M.J.F. Brown • *M. Kelly*

15.00 How disease-ridden are our pollinators? Honeybee pathogens in bumblebees (*Bombus sp.*) across the UK
M.A. Fürst • *M.J.F. Brown*

15.20 Ant workers die young and colonies collapse when fed a high-protein diet
A. Dussutour • *S.J. Simpson*

15.40 The effect of overwintering temperature on the diapause survival, remaining energy resources, and immune functions of *Bombus lucorum* queens
S.R. Vesterlund

16.00 Disruption of trophobiosis between argentine ants and citrus mealybugs: anti-ant strategies adopted by a predator ladybird to eat undisturbed.
D.A. Grasso • *C. Castracani, G. Lazzari, A. Mori*

16.20 Genomic analyses suggest metabolic complementary between fungus-growing termites, their mutualistic fungus and termite gut communities
M. Poulsen • *C. Li, H. Hu, T. Nobre, J. Korb, D. Aanen, J. Boomsma, G. Zhang, J. Wang*

16.40 A call to arms: directional vibration sensing in the termite *Macrotermes natalensis* (Haviland)
F. Hager • *W.H. Kirchner*

17.00 Posters of the second session setup

Wednesday 29

Tuscany Inn Hotel

09.00 PLENARY LECTURE
Social recognition in ants: signature mixtures and identity signals
P. D'ETTORRE

10.00 **Coffee Break**

Parallel Sessions – Oral Communications

FIRENZE room

10.20 Plant selection by leaf-cutting ants: decision-making by foragers and quality control through the symbiotic fungus
F. Roces

10.40 Social learning in bumblebees (*Bombus terrestris*): just attraction to conspecifics or stimulus enhancement?
A. Avarguès-Weber • *L. Chittka*

11.00 The foraging success and organisation of polydomous ant colonies
Z. Cook • *D. Franks, E. Robinson*

11.20 Collective decision making in honeybees: temperature gradient vs social gradient
S. Hahshold • *M. Szopek, G. Radspieler, R.Thenius, T. Schmickl, K. Crailsheim*

11.40 Individual response behavior and collective patterns in bumblebees. How colonies control the temperature of their brood.
A. Weidenmüller

12.00 Nest enlargement in leaf-cutting ants: relocated brood triggers the excavation of new chambers
D. Römer • *F. Roces*

SIENA room

10.20 Resistance to and tolerance of parasitic large blue butterflies by *Myrmica* ant colonies
D.R. Nash

10.40 Comparison of chemical deception strategies and acoustical mimetism between two social parasites of ants
L.P. Casacci • *S. Bonelli, E. Balletto*

11.00 Temporal and spatial patterns of ant social parasite segregation
M. Witek • *L.P. Casacci, F. Barbero, D. Patricelli, M. Sala, E. Balletto, S. Bonelli*

11.20 The social parasitic wasp *P. sulcifer* is not able to suppress host workers ovarian development: what does it tell us about workers reproductive decision?
A. Cini • *L. Dapporto, R. Nieri, T. Monnin, R. Cervo*

11.40 The role of workers and host choice in a fungus growing ant social parasite
J. Larsen • *D.R. Nash*

12.00 Effects of socially parasitic *Myrmica rubra* microgynes on host colonies under laboratory conditions
S. Schär • *D.R. Nash*

The fast and the furious: nest-site selection in the giant Asian honey bee (*Apis dorsata*)
J.C. Makinson • *A. Rattanawannee, B.P. Oldroyd, M. Beekman*

12.20 Should I stay or should I go? Drifting behaviour as a reproductive decision in workers of the bumble bee *Bombus terrestris*
P. Blacher • *S. Chameron, E. Lecoutey, L. Boreggio, P. Devienne, N. Châline*

13.00 **Lunch**

MATING SYSTEMS, DISPERSION AND POPULATION STRUCTURE

Males do not like the working class: evidence of male choice in a social insect
F. Cappa • *C. Bruschini, R. Cervo, S. Turillazzi, L. Beani*

14.20 How *Pyramica sauteri* ants use a novel exocrine gland for prey capture
J. Billen • *C.S. Lam, C.C.Lin*

Mating-induced changes in the behaviour of *Leptothorax gredleri* ant queens
A. Bernadou • *J. Heinze*

14.40 Using multiple factors to classify ant species in the bicolor group (*Cataglyphis* sp. Formicidae:Formicinae)
R. Zeltser • *A. Hefetz*

Genetic polyethism in the polyandrous desert ant *Cataglyphis cursor*
P.A. Eyer • *S. Aron*

15.00 Neuroanatomical study of the antennal lobe of the invasive Asian hornet *Vespa velutina*
A. Couto • *K. Monceau, O. Bonnard, D. Thiéry, J.C. Sandoz*

FUNCTIONAL MORPHOLOGY & SYSTEMATICS

BIODIVERSITY, COMMUNITY ECOLOGY, INVASION BIOLOGY AND IMPACT ON HUMAN AFFAIRS

Loss of flight in insects: the special case of ergatoid queens in ants
C. Peeters

15.20 Comparative molecular and spatial analysis of rare and common bumblebees
S. Dreier • *C. Carvell, A.F.G. Bourke, M.S. Heard, J. Wang, S. Sumner*

Small and large scale implications of independent colony foundation in the European common black ant, *Formica fusca*
H. Johansson • *P. Seppä, H. Helanterä, K. Trontti, L. Sundström*

15.40 Double whammy? Impact of parasites and pesticides on bee fitness
G. Baron • *M. JF Brown, N.E. Raine*

Cryptic structure of native ant supercolonies
P. Seppä • *H. Johansson, N. Gyllenstr, S. Pálsson, P. Pamilo*

16.00 Population genetics of source and reintroduced populations of the socially parasitic Large Blue butterfly *Maculinea arion*
A. Andersen • *L.V. Ugelvig, D.J. Simcox, J.A. Thomas, D.R. Nash*

16.20 **Coffee Break**

Is ejaculate quality in *Apis mellifera* drones affected by senescence, food provisioning and/or immune challenge?
M. Stürup • *J.J. Boomsma, B. Baer*

16.40 How the West Was Won: molecular phylogeny and evolution of the holarctic termites genus *Reticulitermes*
A. Luchetti • *B. Mantovani*

Waste of gametes: Hybridization in the face of strong selection
J. Kulmuni • *P. Pamilo*

17.00 Genetic bottlenecks do not increase invasiveness in natural populations of the invasive garden ant *Lasius neglectus*
R.S. Larsen, J. Mosbacher,L. Holman • ***J.S. Pedersen***

Clonal reproduction and invasiveness in the longhorn crazy ant *Paratrechina longicornis* (Latreille)
M. Pearcy • *M. Goodisman, L. Keller*

17.20 Population genetic structure and range expansion of the tropical fire ant *Solenopsis geminata* in the Galápagos Archipelago
N. Wauters • *W. Dekoninck, F. Hendrickx, D. Fournier*

17.40 Allee effects through caste interaction feedback in ants
G.M. Luque • *F. Courchamp*

18.00 ***SECOND POSTER SESSION***

20.00 **Social Dinner**

Thursday 30

Tuscany Inn Hotel

09.00 PLENARY LECTURE
From bees to computers - nest-site selection in *Apis* spp. as inspiration for optimization algorithms
M. BEEKMAN

10.00 **Coffee Break**

Parallel Sessions – Oral Communications

FIRENZE room

BASIC RESEARCH ON HONEYBEES AND APPLICATIVE OUTPUTS

Persistence to unrewarding feeding locations by forager honey bees (*Apis mellifera*): the effects of experience, resource profitability, and season
H. Al Toufailia • *C. Grüter, F.L.W. Ratnieks*

Seasonal variation in honey bee foraging distance demonstrates critical gaps in food availability
M.J. Couvillon • *F.L.W. Ratnieks*

Genotype-enviroment interactions in the autochthonous Italian peninsula honey bee subspecies (*A.m.ligustica*)
C. Costa • *M. Lodesani, K. Bienefeld*

Emulation of collective honeybee behaviour by a swarm of simple robots
D. Kengyel • *G. Radspieler, F. Wotawa, T. Schmickl*

SIENA room

10.20 Group combats in ants: extending the Lanchester's laws
G. Santini • *F. Frizzi, M. Briffa, F. Bagnoli*

10.40 Dominance hierarchy among highly invasive ants
A. Avril • *C. Bertelsmeier, G.M. Luque, F. Courchamp*

11.00 Hot stuff - Impacts of climate change on invasive ants
C. Bertelsmeier • *G. Luque, B.D. Hoffmann, F. Courchamp*

11.20 Effects of altitudinal gradient on ant community structure in Alpine ecosystems.
F. Spotti • *C. Castracani, D.A. Grasso, A. Mori*

Hygienic behavior in honey bees: influencing factors and behavioral correlates
G. Bigio • *R. Schürch, F.L.W. Ratnieks*

Interspecific within-host competition between *Ascosphaera apis* and *Aspergillus* spp. parasites in honey bee larvae.
K. Foley • *S.E. Evison, P. Chappell, W.O.H. Hughes*

The Trojan Hives: Harmful Pollinator Pathogens, Imported and Distributed in Bee Colonies
P. Graystock • *K. Yates, S. Evison, B. Darvill, D. Goulson, W.O.H. Hughes*

Impact of combined pesticide exposure on individual- and colony-level traits in bees
R.J. Gill • *O. Ramos-Rodriguez, N.E Raine*

11.40 Assessing fragmentation and homogenization in ant communities of southern Mediterranean protected areas (Andalucia, Spain)
E. Angulo, R. Boulay, F. Ruano, A. Tinaut • ***X. Cerdà***

12.00 The influence of urban green area management on ant communities
C. Castracani • *F. Spotti, D.A. Grasso, A. Mori*

12.20 Soil properties weakly affect subterranean ant distribution at small spatial scale
J. Jacquemin • *T. Drouet, Y. Roisin, M. Leponce*

12.40 Ant community structure retains the past for a while... Ant community succession (Hymenoptera: Formicidae) on deciduous forest clear-cuts in Romania
I. Tăușan • *M.R. Trică, L. C. Anghel, A.A. Ștefu, O.T. Bota, A.V. Cravă, B. Markó*

13.00 **Lunch**

FIRST POSTER SESSION - Monday 27 August

MATING SYSTEMS, DISPERSION AND POPULATION STRUCTURE (MAT)

1 • Chemical-physical soil properties of the nesting sites of two syntopic harvester ants: *Messor wasmanni* and *Messor minor* / *D. D'Eustacchio, L. Solida, G. Dowgiallo, D. A. Grasso, A. Fanfani*

2 • Hybridogenesis through thelytokous parthenogenesis in two *Cataglyphis* desert ants / *Pierre-André Eyer, Laurianne Leniaud, Hugo Darras, Serge Aron*

3 • Life-history traits as causes or consequences of polygyny / *Dóra B. Huszár, Jes Søe Pedersen*

4 • No inbreeding depression but increased investment in sexual reproduction in highly inbred ant colonies / *Ilka M. Kureck, Evelien Jongepier, Beate Nicolai, Susanne Foitzik*

5 • The proximate effects of temperature on bumble bee colony longevity and the foundation of a second annual generation / *Jacob G. Holland, Andrew F. G. Bourke*

6 • Do *Formica fusca* queens compete through egg quality? / *Martina Ozan, Heikki Helanterä, Liselotte Sundström*

7 • Reproductive behaviour of the honey bee population in Ile de France: Influence of the colonies in drone congregation set up / *Bénédicte Bertrand, Sibyle Moulin, Hélène Legout, Mohamed Alburaki, Lionel Garnery*

DISEASES, IMMUNITY, SYMBIONTS AND SOCIAL PARASITES (IMM)

1 • Antiseptic homes: from solitary to advanced eusocial wasps the nest turns into a social immunity tool / *David Baracchi, Giuseppe Mazza, Mario Polsinelli, Stefano Turillazzi*

2 • How internal state and social cues affect colony preference in the context of social parasitism in the bumblebee *Bombus terrestris* / *Laurie Boreggio, Pierre Blacher, Paul Devienne, Nicolas Châline, Stéphane Chameron*

3 • Lack of evidence for local adaptation suggests uniform coevolution in a paper wasp social parasite-host system / *Alessandro Cini, Rachele Nieri, Rita Cervo*

4 • Ant community structure of syntopic populations of *Maculinea alcon* and *Maculinea 'rebeli'* (Lepidoptera: Lycaenidae) / *Zsolt Czekes, Gyöngyvér Molnár, Márta Ferencz, Bernadette Lázár, Bálint Markó*

5 • Factors conditioning the success of the different individual reproductive strategies available to bumble bee workers / *Labédan Marjorie, Blacher Pierre, Boreggio Laurie, Devienne Paul, Poteaux Chantal, Chameron Stéphane, Châline Nicolas*

6 • Association of *Aphaenogaster subterranea* (Hymenoptera: Formicidae) with the nymphs of *Reptalus panzeri* (Hemiptera: Cixiidae) / *Gábor Lőrinczi*

7 • Selective response of *Formica cinerea* Mayr (Hymenoptera: Formicidae) to corpses of different origin / *István Maák, Bálint Markó, Katalin Erøs, Hanna Babik, Piotr Slipinski*

8 • Examining the "evolution of increased competitive ability" hypothesis in response to co-evolved and general parasites in the invasive paper wasps *Polistes dominulus* / *Fabio Manfredini, Laura Beani, Christina M. Grozingeri*

9 • Evolution of immune genes in the invasive Argentine ant / *Jenni Paviala, Lumi Viljakainen, Jes S. Pedersen, Heikki Helanterä*

10 • Facultative endosymbionts influence on sugar and amino acid composition of honeydew *Aphis fabae* / *Schillewaert S., Parmentier T., Vorburger C., Wenseleers T.*

11 • Analysis of an Exon Cassette of a Hyperdivers Immune Gene in Ants / *Ellen A. Schlüns, Helge Schlüns*

12 • Microbial activity in wood ant nest and its role in ant thermoregulation and nutrient cycling / *Jan Frouz, Veronika Jilkova*

CHEMICAL ECOLOGY (CHEM)

1 • Recognition of nests and nestmates in the African stingless bee *Hypotrigona gribodoi* / *Kathrin Krausa, Jana Wolf, Wolfgang H. Kirchner*

2 • Hymenopteran birth control: ants and wasps share conserved class of sterility-enforcing queen pheromones / *Annette Van Oystaeyen, Luke Holman, Hilde Huyghe, Carmen Romero, Falko Drijfhout, Jocelyn Millar, Patrizia d'Ettorre, Johan Billen, Tom Wenseleers*

3 • Comparison of cuticular hydrocarbons profiles among nature and produced workers and queens in the stingless bee *Frieseomelitta varia* (Hymenoptera, Apinae, Meliponini) and acceptance of the new queen / *Ana Rita T. O. Baptistella, Maria Juliana Ferreira-Caliman, Fábio S. Nascimento, Ademilson E. E. Soares*

4 • Behavioural interactions between larvae and workers in ants: the role of larval odours in stress situation / *Aurélie Daumas, Renée Fénéron, Gilles Gheusi, Fabrice Savarit*

5 • How colony nutritional state affects individual response thresholds to larvae in ants / *Catherine Geay, Fabrice Savarit, Paul Devienne, Renée Fénéron*

6 • Silenced by the regime: honeybee workers exposed to virgin queens develop ovaries but fail to advertise fertility / *Orlova Margarita, Malka Osnat, Hefetz Abraham*

7 • Chemical correlates of male fighting in an ant species with a male diphenism / *Antonia Klein, Jürgen Heinze, Jan Oettler*

8 • Do environmental factors influence social organization traits in *Reticulitermes* termites? / *L. Lefloch, A-G. Bagnères, S. Dupont, J-P. Christidès, C. Lucas*

9 • Population analysis of *Crematogaster scutellaris* colonies: combining behavioural, genetic and chemical information / *Filippo Frizzi, Leonardo Dapporto, Claudio Ciofi Guido Chelazzi, Stefano Turillazzi,Giacomo Santini*

10 • Does Cuticular Hydrocarbon composition explains acceptance and rejection behaviour in the honeybee? / *Francesca R. Dani, Duccio Pradella, Steven J. Martin*

GENES, GENOMES AND SOCIAL BEHAVIOUR (GEN)

1 • Genes behind chemical communication? – Rapid evolution and positive selection in the CSP gene family of ants / *Jonna Kulmuni, Yannick Wurm, Pekka Pamilo*

2 • The role of microRNAs in caste determination and differentiation in the eusocial insect *Bombus terrestris* / *David H. Collins, Matthew Beckers, Tamas Dalmay, Andrew F.G. Bourke*

3 • The genetic basis of reproductive division of labor in *Temnothorax* ants: First results of a gene expression study / *B. Feldmeyer, S. Foitzik*

4 • The expression of aging: gene-expression differences underlying the disparity in lifespan between queen and worker ants / *Eric R. Lucas, Oksana Riba-Grognuz, Miguel Corona, Yannick Wurm, Laurent Keller*

5 • miRNAs ame-miR-9c, ame-miR-3747b and ame-miR-306 are potential regulators of EcR, usp and ftz-f1 expression in *Apis mellifera* queen ovaries / *Liliane MF Macedo, Tathyana RP Mello, Karina R Guidugli-Lazzarini e Zilá LP Simões*

6 • Variation in caste specific Vitellogenin expression within and among colonies of *Formica* ants / *Morandin Claire, Jes S. Pedersen, Heikki Helanterä*

7 • Signatures of divergence and selection in *Cardiocondyla obscurior* / *Lukas Schrader, Jürgen Heinze, Jürgen Gadau, Jan Oettler*

8 • Intra-organismal competition in chimeric colonies of the clonal ant *Cerapachys biroi*/ *Teseo S., Châline N., Jaisson P., Kronauer D.J.C*

9 • A Y-like social chromosome causes alternative colony organization in fire ants / *Yannick Wurm, John Wang, Mingkwan Nipitwattanaphon, Oksana Riba-Grognuz, DeWayne Shoemaker, Laurent Keller*

10 • Sex specific gene expression and haplodiploidy purging / *Kalevi Trontti, Heikki Helanterä, Christopher Wheat*

SECOND POSTER SESSION - Wednesday 29 August

FUNCTIONAL MORPHOLOGY SYSTEMATICS (MOR)

1 • Functional morphology of the mandibular gland of queens of the ant *Monomorium pharaonis* (L.) / *Sofie Boonen, Dieter Eelen, Lisbeth Børgesen, Johan Billen*

2 • Evolution of the frontal gland in termite imagoes / *Katerina Kutalová, Thomas Bourguignon, David Sillam-Dussès, Robert Hanus, Yves Roisin, Jan Sobotník*

SOCIAL BEHAVIOUR AND COLLECTIVE PROCESSES IN SOCIAL INSECTS (SOC)

1 • Collective decision making of young honeybees in complex thermal environments / *Martina Szopek, Gerald Radspieler, Ronald Thenius, Thomas Schmickl, Karl Crailsheim*

2 • Division of labour among trunk-trail workers of the harvester ant *Messor wasmanni* / *Solida L., Castracani C., Mori A., Fanfani A., Grasso D.A*

3 • Collective operant conditioning in three *Myrmica* species / *Marie-Claire Cammaerts, David Cammaerts, Zoheir Rachidi, Stéphanie Némeghaire*

4 • Behavioral profile and guiding efficiency of group leaders in the pavement ant *Tetramorium caespitum* / *Collignon B., Cervantes Valdivieso L.E., Detrain, C*

5 • Short-term effects of changing food conditions on the polydomous nest-network of *Formica lugubris* in England / *Samuel Ellis, Elva Robinson*

6 • The evolution of division of labour in primitive and advanced ants / *Henry Ferguson-Gow, Kate Jones, Seirian Sumner, Andrew Bourke*

7 • How *Myrmica rubra* foragers find their way while foraging? / *Marie-Claire Cammaerts*

8 • Ant activity and temperature regime in nests of wood ants *Formica polyctena* / *Stepánka Kadochová, Jan Frouz*

9 • The coexistence of the plesiobiotically associated colonies of *Formica fusca* and *Camponotus vagus* / *Orsolya Kanizsai, László Gallé*

10 • Slavemaker select for high aggression in host colonies / *Tobias Pamminger, Isabelle Kleeberg, Mirjam Papenhagen, Susanne Foitzik*

11 • The "SOS" in *Cataglyphis cursor* ants / *Céline Amirault, Pyrène Rieu, Chloé Leroy, Jean-Luc Durand, Karen L. Hollis, Alain Lenoir, Elise Nowbahari*

12 • *Cataglyphis cursor* ants are able to discriminate between individual nestmates / *Oliver Delattre, Elise Nowbahari*

13 • Interindividual variability in sucrose response thresholds and division of labor in the ant *Camponotus aethiops* / *Margot Perez, Martin Giurfa, Patrizia d'Ettorre*

14 • Facial markings as quality signal in the hover wasp *Liostenogaster vechti* (Hymenoptera Stenogastrinae) / *Baracchi D., Petrocelli I., Cusseau G., Pizzocaro L., Teseo S., Turillazzi S.*

15 • Brood presence and available space influence digging activity and lead to nest-size adjustment in leaf-cutting ants / *Daniela Römer, Flavio Roces*

16 • Extremely male-biased sex-ratio in a socially polymorphic sweat bee (*Halictus rubicundus*) / *Roger Schürch, Jeremy Field*

17 • Antennal drumming in the primitive eusocial wasp *Mischocyttarus* (*Megacanthopus*) *parallelogrammus* (Hymenoptera, Vespidae) / *Olga Coutinho Togni, Edilberto Giannotti*

18 • The effect of social isolation on physiology, behaviour and fitness in ants / *Akiko Koto, Laurent Keller*

19 • Articulate communication with an unintelligible vocabulary / *Nitzan Razin, Jean-Pierre Eckmann, Ofer Feinerman*

20 • When worlds collide / *Daphna Gottlieb, Ana Sendova-Franks, Nigel R. Franks*

21 • Individual reproductive benefits shape labour division in the ant Platythyrea punctata and the ant Pseudomyrmex gracilis / *A. Graclik, A. Langowska, O. Wasielewski, P. Tryjanowski, B. Walter*

BIODIVERSITY, COMMUNITY ECOLOGY, INVASION BIOLOGY AND IMPACT ON HUMAN AFFAIRS (ECO)

1 • Phylogenetic analysis of the community structure of southern African termites / *Janine Schyra, Judith Korb*

2 • Close relationships between the yeast *Saccharomyces cerevisiae* and social wasps / *I. Stefanini, L. Dapporto, JL. Legras, A. Calabretta, M. Di Paola, C. De Filippo, P. Capretti, M. Polsinelli, S. Turillazzi, D. Cavalieri*

3 • Temporal changes of myrmecochory by the red ant *Myrmica rubra* / *Bologna A., Detrain C.*

4 • Interference of Social Insects on Human Affairs / *Davide Di Domenico, Alessandro Bione, Bruno Baldassarre, Manuela Travaglio, Alberto Zotti, Lara Maistrello*

5 • Influence of forage sources on *Andrena* species richness and abundance in meadows / *L. Fortunato, M. D'Agaro, P. Zandigiacomo*

6 • Effects of overwintering temperature on the survival of garden ant *Lasius niger* / *Marja-Katariina Haatanen, Jouni Sorvari*

7 • The value of a holy forest for Nature conservation: ants in a refuge / *Jean Jacques Itzhak Martinez, Ziv Amar, Armin Ionescu-Hirsch*

8 • The Italian School of Ants / *Fiorenza Spotti, Cristina Castracani, Donato A. Grasso, Alessandra Mori*

BASIC RESEARCH ON HONEYBEES AND APPLICATIVE OUTPUTS (BEE)

1 • Honeybee workers' reproduction and their life expectancy / *Michal Woyciechowski, Karolina Kuszewska, Anna Woloszczuk*

2 • Behavioural reversion in honey bee workers with different life expectancy / *Karolina Kuszewska, Michal Woyciechowski*

3 • Members of molecular signaling pathways underling hind leg development in honeybee castes / *Bomtorin, AD; Barchuk, AR; Cristino, AS; Laure, MAFB; Simoes, ZLP*

4 • On the transmission strategies of the parasitic mite *Varroa destructor* among beehives / *R. Cervo, C. Bruschini, F. Cappa, S. Meconcelli, G. Pieraccini, D. Pradella, S. Turillazzi*

5 • Evidence for a negative correlation between the honey bee pathogens *Nosema ceranae* and Deformed Wing Virus / *Cecilia Costa, Gina Tanner, Marco Lodesani, Lara Maistrello, Peter Neumann*

6 • The APENET project: a multidisciplinary approach / *M. Lodesani, L. Bortolotti, C. Costa, P. Medrzycki, C. Porrini*

5th Meeting of the European Sessions of the I.U.S.S.I.

Montecatini Terme
26-30 August 2012

Abstracts[1]

[1] *The text of the abstracts of all the contributes has been left unchanged from that presented by the authors. Only the format has been edited to reach uniformity*.

Plenary lectures

Towards an integrated understanding of social behaviour

Seirian Sumner

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A key innovation in social evolution is the emergence of behavioural commitment by individuals into reproductive or non-reproductive castes. How does such phenotypic variation arise and what are the fitness consequences of such commitment? Like most plastic phenotypes, social insect castes usually arise in response to the environment with coordinated changes in gene expression that have been selected over evolutionary time to maximise fitness. A holistic study of social phenotypes, linking the fundamental units of life - the genes - to the phenotypic changes (and fitness consequences) they underlie, is a powerful new approach made possible by a new era of accessible genomic methodologies . The field of sociogenomics has significantly enhanced our understanding of social behaviour, particularly regarding the molecular mechanisms regulating them. However, mechanisms are only one facet of behaviour. Niko Tinbergen famously defined four key lines of inquiry for behavioural ecologists seeking to understand variation in behaviour, from proximate (machinery and development) to ultimate (shared evolutionary history and adaptive value) factors. For the first time, we are now able to examine each of these facets from gene to phenotype, adding an important new dimension to the study of social behaviour. I discuss how combining genomics with the classical behavioural ecology approach can bring us towards a clearer understanding of social behaviour, within Tinbergen's framework. The *Polistes* paper wasps have long been a behavioural model system for understanding the origins of sociality. I discuss how this genus is now emerging as a key socio-genomics model for understanding the early stages of social evolution and caste commitment from gene to phenotype. Using examples from our recent work on *Polistes*, I will argue how an integrated approach of genomic, transcriptomic and epigenetic analyses with behavioural can reveal facets of social behaviour and social evolution that we could not previously discern, bringing us closer to understanding both the proximate and ultimate factors underlying social behaviour.

Collective disease defence in ant societies

Sylvia Cremer

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Encountering a diseased individual can elicit diverse behaviours ranging from avoidance to intensive care. Colonies of ants take very good care of their pathogen-exposed nestmates, and have evolved collective disease defences at the behavioural, physiological and organisational level, i.e. a “social immune system”. We study how pathogen exposure of individual ants affects their individual behavioural and physiological defences, and also the reaction of healthy nestmates. Social interaction networks change after exposure due to alterations in e.g. grooming behaviour of both the diseased ant and its nestmates. Whereas allogrooming reduces the chance of exposed individuals to get a deadly infection, it can also induce pathogen spread to helping nestmates. Interestingly, this socially induced pathogen transfer does not necessarily make nestmates sick, but can also give rise to specific immune gene upregulation and protective immune stimulation if infection levels are low. Allogrooming of fungal pathogens is particularly efficient before the spores penetrate the cuticle and cause internal infections in their host. Ants are indeed able to detect the presence of spores before infections establish and perform antiseptic behaviours immediately after exposure. The basis of this early detection is little understood, but its effect in curing exposed individuals and keeping epidemics in check is likely very high. We try to determine the selection pressures of collective immune defences on the evolution of pathogens coevolving with societies and use epidemiological modelling to determine the effects of different components of social immunity on disease dynamics.

Social recognition in ants: signature mixtures and identity signals

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Communication of identity allowing recognition of group members is essential for the evolution and stability of social life. Social insects use primarily the chemical channel for communicating social identity through multi-component cues and signals. In ants, and other social insects, social interactions are regulated by at least three levels of recognition. Nestmate recognition occurs between colonies, is usually very effective, and involves fast processing of signature mixtures (blends of cuticular hydrocarbons). Cuticular hydrocarbons differ in structure, with consequences on their perception, functional role and heritability. Within a colony, division of labour is enhanced by recognition of different classes of individuals. Pheromones are involved in signalling reproductive status and regulating reproductive conflicts. In some cases, a single hydrocarbon can act as queen pheromone conveying honest information about quality and controlling worker reproduction. Ultimately, in particular circumstances, such as cooperative colony founding with stable dominance hierarchies, ants are capable of chemically based individual recognition. Social context can help predicting recognition systems, and the underlying recognition cues and mechanisms appear to be specific to each recognition level. Their integrated understanding can contribute to the understanding of social systems and their evolutionary stability.

From bees to computers - nest-site selection in *Apis* spp. as inspiration for optimization algorithms

Madeleine Beekman

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During reproductive swarming and seasonal migration, a honeybee swarm needs to locate and move to a new nest site. Our current knowledge of swarming and nest-site selection in honeybees is based primarily on studies of just one species, *Apis mellifera* (reviewed in (Visscher 2007). Natural colonies of *A. mellifera* live in tree cavities. The quality of the cavity is often critical to the survival of a swarm. The scouts must search thousands of trees to ensure that they do not settle for a poor cavity when a better one is available.

Recently we started to investigate how swarms of the open-nesting dwarf honeybee *Apis florea* selects a new home (Makinson et al. 2011; Oldroyd et al. 2008). For a cavity-nesting species like *A. mellifera* there is only a limited number of potential nest sites that can be located by a swarm. In contrast, for an open-nesting species like *A. florea* which builds a small nest comprised of a single comb suspended from a twig of a shrub or tree in the open (Oldroyd and Wongsiri 2006), it seems that there is an abundance of shaded twigs that would be equally suitable for building a nest. Our work showed that the nest-site selection process of *A. florea* and *A. mellifera* are shaped by each species' nest-site requirements (Diwold et al. 2010; 2011b; Schaerf et al. 2011).

Contrary to *A. mellifera*, nest-site selection in *A. florea* appears to be more similar to search-space sampling than to a decision-making process. Bees scout the environment for general areas in which potential nest sites are abundant. We therefore proposed that the bees' nest-site selection process can be used as a basis for the development of new bee-inspired optimization algorithms (Diwold et al. 2010). The first such algorithm has recently been developed (Diwold et al. 2011a).

Diwold K., Beekman M., Middendorf M.. 2010. Bee nest site selection as an optimization process. In: Fellerman H, Dörr M, Hanczyc MM et al. (eds) Artificial Life XII. Proceedings of the twelfth international conference on the synthesis and simulation of living systems. The MIT Press, pp 626-633

Diwold K., Himmelbach D., Meier R., Baldauf C., Middendorf M.. 2011a. Bonding as a swarm: applying bee nest-site selection behaviour to protein docking. Paper presented at the Proceedings of the 13th annual conference on Genetic and evolutionary computation, Dublin, Ireland,

Diwold K., Schaerf T.M., Myerscough M.R., Middendorf M., BeekmanM.. 2011b. Deciding on the wing: in-flight decision making and search space sampling in the red dwarf honeybee *Apis florea*. Swarm Intell 5:121-141

Makinson J.C., Oldroyd B.P., Schaerf T.M., Wattanachaiyingchareon W., Beekman M.. 2011. Moving home: nest site selection in the Red Dwarf honeybee (*Apis florea*). Behavioral Ecology and Sociobiology 65 (5):945-958

Oldroyd B.P., Gloag R.S., Even N., Wattanachaiyingcharoen W., Beekman M.. 2008. Nest-site selection in the open-nesting honey bee *Apis florea*. Behavioral Ecology and Sociobiology 62 (10):1643-1653

Oldroyd B.P., Wongsiri S.. 2006. Asian honey bees. Biology, conservation and human interactions. Harvard University Press, Cambridge, MA

Schaerf T.M., Makinson J.C., Myerscough M.R., Beekman M.. 2011. Inaccurate and unverified information in decision making: a model for the nest site selection process of *Apis florea*. Animal Behaviour 82:995-1013

Visscher P.K.. 2007. Group decision making in nest-site selection among social insects Annual Review of Entomology 52:255-275

Session 1. Social behaviour and collective processes in social insects

Social and spatial organization in an ant colony

Danielle Mersch [1], Alessandro Crespi [2], Laurent Keller [1]

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Ant colonies are highly organized social organisms with efficient division of labor among workers. However, despite many studies on division of labor, little is known about the social and spatial organization of ant colonies. Tracking the social interactions and spatial movement of 804 individually labeled *Camponotus fellah* ants from six colonies continuously for 41 days revealed that workers organize in three socially cohesive groups in which workers interact up to eight times more often than between groups. Each social group almost perfectly matched a distinct behavioral task: brood care, nest patrolling, and foraging. Comparison of the normalized age of workers of the three groups revealed temporal polyethism with nurses that were on average younger than nest patrollers, which in turn were younger than foragers. Tracking individual group switches over time among the three behavioral groups further showed that workers switched groups non randomly moving from brood care to nest patrolling, and from nest patrolling to foraging. Spatial heat maps further revealed that the social groups strongly segregated in space thereby generating a spatial structure in a nest that was originally devoid of any landmarks, except the nest entrance. All together our results show that ant colonies are organized in several interconnected social groups that strongly segregate in space, and that each of the group is specialized in a specific task, thereby embedding division of labor in the spatial and social structure of an ant colony.

A Field Study and Computer Simulation Demonstrate how Colony Size and Food Distribution Affect Ant Foraging Rates

Melanie E. Moses [1, 2, 3], Tatiana P. Flanagan [1, 2], Kenneth Letendre [1, 2]

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[2]Department of Computer Science, University of New Mexico Albuquerque, NM USA
[3]The Santa Fe Institute, Santa Fe, NM USA

Desert seed-harvester ants in the genus *Pogonomyrmex* are central place foragers that search for resources collectively. We conducted a field study in the central New Mexico desert and a computer simulation to understand how ants use information about the distribution of seeds in the environment to improve foraging success. We quantify how much faster seed harvesters collect seeds in clumped versus scattered distributions. A complementary computer simulation demonstrates how a combination of individual memory (site fidelity) and pheromone communication maximize foraging rates and replicate foraging patterns of ants in the field study. We find that foraging rates are significantly influenced by the spatial distribution of experimental seed baits. Colonies collect seeds from larger piles faster than randomly distributed seeds—foraging rates double when seeds are clumped into piles four times bigger. We explain this result based on the greater information obtained, remembered and communicated by ants when they encounter larger piles. The increase in foraging rate when food is clumped in larger piles is indistinguishable across three species that vary from a few dozen to a few thousand foragers. This suggests that larger colonies are no better than smaller colonies at collecting clumped seeds, in contrast to the theoretical expectation that larger groups are more efficient at exploiting clumped resources

Selection on colony personalities

Andreas P. Modlmeier, Tobias Pamminger, Susanne Foitzik

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Division of labour is a key feature of insect societies for which intracolonial behavioural variation is essential. As the fitness consequences of animal personalities in social insects are largely unexplored, we tested how behavior and its variation on the individual and the group level influenced colony productivity and survival in the ant *Temnothorax longispinosus*. First, we investigated intercolonial variability and its consistency across time and contexts in aggression, exploration and brood care behavior. Aggressiveness was consistent over several months even after the emergence of a new worker generation, while other behaviours were inconsistent over time. Exploration responded to the trial sequence: colonies were faster in discovering when workers previously encountered opponents in aggression experiments. Suites of correlated behaviours (e.g. aggression – exploration syndrome) did not persist over time. Most importantly, colonies with higher intracolonial behavioural variation in brood care and exploration were more productive under standardized conditions than colonies with less variation.
In a second trial series we questioned whether these collective personality traits are under selection in the field. In general, parasite pressure leads to the evolution of sophisticated defense strategies in hosts of slavemaking ants. We have previously shown that *Temnothorax longispinosus* ant colonies become more aggressive with increasing nest density and after contact to the slavemaking parasite, *Protomognathus americanus.* In a field manipulation, we aimed to disentangle the impact of parasite presence and nest density on host aggression. An early slavemaker mating flight provided the unique opportunity to study the influence of host aggression on the founding success of slavemaking queens and their dispersal strategies. Parasitic queens avoided colony foundation in parasitized areas indicating that they either evade intraspecific competition or fail to take-over host colonies in parasitized locales. Furthermore, parasite queens captured more brood from less aggressive host colonies indicating that the slavemaker founding phase selects for a certain collective behavioral type, ie. more aggressive host colonies.

Caste-biased gene expression underlying division of labour and dominance in a Primitively Eusocial Ant: the role of differential gene expression

Claire Asher [1, 2]*, Pedro Ferreira [3], Heinz Himmelbauer [3], Roderic Guigo [3], Fabio Nascimento [4], William Hughes [2], Seirian Sumner [1]

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3 *Centre for Genomic Regulation,* Doctor Aiguader, 88, 08003 Barcelona, Spain.
4 Departmento de Biologia, FFCLRP, Universidade de Sao Paulo

The huge success of ants can be largely attributed to division of labour, which increases colony efficiency and productivity (1). Whilst lacking morphological castes, primitively eusocial species, such as ponerine ants, show behavioural division of labour, both in reproduction and other colony tasks (e.g. foraging). In contrast to more advanced eusocial species (and indeed most other ants), ponerine ant workers retain reproductive totipotency – the ability to switch caste from non-reproductive (worker) to reproductive (gamergate) (2). A portion of the colony forms a linear hierarchy which will determine the identity of the next reproductive (gamergate) (2). This hierarchy is maintained using a combination of ritualised aggressive interactions and cuticular hydrocarbon signals (3). Individuals within a colony vary in their chances of obtaining direct fitness in the future, and this has important consequences for their behaviour. The behavioural plasticity in the adults of these ants is secondarily derived, and therefore presents an exciting opportunity to compare with the behavioural plasticity found in primitively eusocial bees and wasps. Behavioural differences between genetically and morphologically identical individuals are usually caused by differential expression of shared genes. Using a mixture of behavioural observations and radio frequency identification (RFID), we investigate the effect of rank on behaviour in the primitively eusocial ponerine ant, *Dinoponera quadriceps*. We analyse these behavioural attributes in the context of phenotype-specific transcription data, derived from next-generation genome and transcriptome sequencing, to reveal the relationship between genes and behaviour. We show a strong affect of dominance rank on behaviour in *Dinoponera quadriceps*, and relate this to key genes whose expression is related to rank. We compare the genes associated with behavioural castes in *D. quadriceps* with those identified in other eusocial insects in order to determine the extent to which a genetic toolkit for social behaviour is conserved.

1) Wilson, E. 1974. The Insect Societies
2) Peeters, C. 1991. Biol J Linn Soc. 44: 141 - 152
3) Peeters, C *et al*. 1999. P Roy Soc Lond B Bio. 266: 1323 - 1327

Division of Labor in *Cataglyphis cursor* Ants Determines Who Performs Precision Rescue Behavior

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Recently, we reported that *Cataglyphis cursor* ants use sophisticated rescue behavior to extricate nestmates that have become ensnared by collapsing sand or debris (Nowbahari et al., 2009). In addition to limb-pulling and sand-digging behavior, ants were able to identify the snare that bound their nestmate, to transport sand away from it, and then to target their bites precisely to it alone, excavating sand as necessary. Here we present evidence that this behavior is regulated by a division of labor, which determines who can administer – and receive – help. We manipulated both the caste of the rescuers and the caste of the victim in a two-way factorial design: Each group of 5 rescuers contained members of only one caste (foragers, nurses, or inactives) and each of these caste-rescuer groups was paired with a single caste victim (a forager, nurse or inactive), for a total of 9 different rescuer-victim combinations. In addition, control tests were conducted in which the victim was anesthetized by chilling. The results were that members of the forager caste administered and obtained the most help while members of the youngest (inactive) caste not only were incapable of responding to entrapped nestmates, but also received virtually no help from potential rescuers, regardless of caste. Nurses performed intermediate levels of aid, mirroring their intermediate caste status. Our results indicate that division of labor, a form of *temporal polyethism* in which individuals engage in different tasks as they mature, also controls their ability to give and receive the kind of precision rescue behavior of which this species is capable.

Nowbahari, E. et al. 2009. *PLoS ONE 4(8)*: e6573.

Reduction of potential fecundity leads to premature foraging in ants – an ancestral trait in in the evolution of eusociality?

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The enormous evolutionary success of eusocial insects (ants, bees, wasps, termites) was possible due to precise labour division into reproductives, nurses and foragers. Though intensively studied for decades, the ultimate and proximate mechanisms underlying the evolution of labour division are still not fully understood. In the study I examined (1) whether less fecund individuals become forages, while more fecund individuals remain in the nest as nurses irrespectively to their age, and, (2) whether the phenomenon is constant in diverse lineages of ants (*Ponerinae, Pseudomyrmecinae, Myrmecinae, Formicinae*). As study systems I used species with totipotent individuals (*Platythyrea punctata*), as well as species with distinct worker caste (*Pseudomyrmex gracilis, Temnothorax unifasciatus*, *Myrmica scabrinodis, Formica rufibarbis*) where workers start to reproduce as soon as the queen is dead or lost. First, I compared fecundity of foragers vs. nurses of unknown age in unmanipulated colonies. Second, I examined fecundity of forages vs. nurses in cohorts where all individuals were of the same age. Third, I manipulated fecundity of individuals and observed whether individuals with reduced fecundity become forages and individuals with increased fecundity become nurses. The survival of forages and nurses in all experiments was examined to exclude an effect of life expectancy reduction on labour division. The results showed that reduction of fecundity leads to precocious foraging, while increase of fecundity results in reverse to nurse and that new forages have decreased fecundity already on their first foraging run. This shows that individuals with decreased fecundity are more likely to become foragers which decreases their chances to reproduce even further. The division was constant in all examined ant subfamilies irrespectively of individual age and life expectancy indicating an ancestral trait in evolution of labour division and potentially one of the key elements that led to origin of eusociality.

Low relatedness can favour the evolution of eusociality

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Recently, the effect of relatedness as a driving force for the evolution of eusociality has been questioned, giving rise to a heated debate between skeptics and supporters of kin selection theory. A complete resolution of the debate, however, is not possible given that most of the existing models on the evolution of eusociality either make highly unrealistic assumptions or do not model costs and benefits in any level of detail. Hence, we here construct a biologically more realistic model of the evolution of eusociality. We do this by considering the invasion and maintenance of a mutant helping allele in a population of solitary insects. We assume that up until a critical switching time in the season, carriers of the eusocial allele display worker behaviour and help with the rearing of siblings, but that individuals born after that will disperse and mate population-wide. Colony growth of eusocial colonies is assumed to conform to a delayed logistic growth model. Finally, genetic relatedness is varied by considering different queen mating frequencies. Surprisingly, our model predicts that eusociality invades more easily under multiple mating (low relatedness) than under single mating. This effect arises because multiple mating causes relatively more family groups to contain at least a small fraction of workers, and in our model even a small fraction of workers is sufficient to yield a productive colony -- a situation known as "behavioural dominance". When analyzed from an inclusive fitness angle, the results from our model can also be understood in terms of costs and benefits being a non-linear function of mating frequency. Rather than showing that kin selection theory is not useful for explaining the evolution of eusociality, our results show that previous models have ignored important effects, such as costs and benefits being dependent on relatedness, even though these are very likely to occur in nature.

Cannibalize this: a guide to selfishness for ant larvae

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The polyphenic development into reproductive queens and sterile workers is a fundamental property of social insect colonies. In such systems, conflict over caste determination can arise because individuals gain direct fitness benefits by developing into queens. Selection would therefore favor larvae displaying selfish traits which increase chances of turning into a queen. Egg cannibalism is a prime example of such a selfish trait. In ants, brood is reared in batches and larvae commonly feed on eggs, a high-quality food source readily available within the nest. As caste fate is often influenced by nutrition, cannibalistic larvae could increase their chances of developing into queens, while at the same time removing competitors. In mediating this conflict, the relatedness between colony members is crucial because individuals would suffer severe indirect fitness losses from cannibalizing kin. As a result, the intensity of cannibalism is predicted to increase when relatedness between nestmates is low, for example in colonies with multiple queens. We investigated larval egg cannibalism in the ant *Formica aquilonia*, an ideal system for studying the effects of relatedness on selfishness since queen numbers can be extremely high. However, when comparing broods with experimentally manipulated levels of genetic diversity, we found no difference in the intensity of cannibalism. Furthermore, analysis of the chemical profiles of eggs laid by different queens indicates that while information on relatedness may be available, larvae do not seem to use these cues to adjust their behavior.

Unexpected fusion of genetically divergent colonies in the invasive pharaoh ant

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Colonies of the highly invasive pharaoh ant *Monomorium pharaonis* have low genetic diversity and are extremely genetically divergent, even within tropical populations where colonies are spatially close and free to move (Schmidt et al. 2010). This suggests that local mixing even of related colonies is very limited, and that budding often leads to new isolated lineages. Paradoxically, the ant shows low discrimination of nestmates raising the question whether genetic differentiation is sufficient to prevent unrelated colonies to fuse. In this study, eight highly genetically divergent lineages were sequentially crossed following the scheme of Hansen and Spuhler (1984) in order to obtain a genetically heterogeneous stock. Experimental colonies (n = 16) were chosen from this breeding scheme and paired at three levels of genetic similarity (relatedness) in a fusion assay with queens, workers and brood in nest boxes connected by a common foraging arena (cf. Vásquez and Silverman 2008). Each colony was marked by feeding it with a water soluble dye, and the distribution and social interactions of the paired colonies (n = 6 replicates) were followed for 14 days. Furthermore, we analysed how colony genetic composition and cuticular hydrocarbons affected the outcome. We found that most pairs of colonies merged within 4 days only, even if colonies were genetically highly divergent and had common fighting of workers in the beginning of the experiment. This shows that the low level of nestmate discrimination generally found in the pharaoh ant has the potential to lead to fusion of colonies, thus restoring genetic diversity. Why such fusion appears not to occur in natural populations of spatially close colonies is currently unknown but could be due to environmental factors.

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Colony fusion and reproductive conflicts in *Temnothorax* ants

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According to social evolution theory, stable altruism can evolve if it is aimed at related individuals, thus conferring substantial indirect fitness benefits (*kin selection*). Eusocial Hymenoptera, which present high levels of relatedness among colony members, are often considered as textbook examples of kin selection. However, altruistic behaviour towards non-kin has also been reported, and this deserves closer examination. In ants, for example, alien colonies occasionally merge into a single cooperative unit. Such fusions can occur after queen loss and may contribute to increase orphaned workers' inclusive fitness if merging colonies are related (Kronauer et al. 2010). In the monogynous, seasonally polydomous ant *Temnothorax nylanderi*, however, fusions can also involve two queenright colonies and result in the death of one queen, at considerable costs for her worker offspring (Foitzik and Heinze 1998; Strätz et al. 2002). We investigated the dynamics and outcome of nest fusion among queenless and/or queenright colonies in *T. nylanderi*, and tested whether fused colonies function as homogeneous units. Using the reunion of fragments from the same mother colony as a control, we found that fusions between alien colonies were significantly delayed due to aggression among non-nestmates. Nevertheless, nest fusions between queenless colonies had a similar success rate as reunions between nestmate fragments, and were more likely to succeed than fusions involving at least one queenright colony. This suggests that queen presence increases workers' incentive to preserve colony integrity. In addition, workers from merging colonies remained spatially segregated within the nest after fusion, thus possibly retaining the ability to split again according to kinship. Our data also revealed interesting seasonal effects: colony fused more readily in spring than in summer. Ongoing behavioural, chemical and genetic analyses will help understand the full significance of these observations in terms of fitness for merging colonies.

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Alien queen acceptance in queen-rich and queenless colonies of ant *Myrmica rubra*

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Alien conspecific queens are sometimes accepted in polygynous ant species (e.g., Holzer B. et al. 2008. Ins. Soc. 55: 392-396). Polygynous and polydomous red ant *Myrmica rubra* often establishes satellite nests during summer. Many of such satellite nests are queenless in the beginning. It has been noted earlier that queenless colonies of *M. rubra* can accept alien queen of the same species (Elmes GW., Brian MV. 1991. Ins. Soc. 38: 51-62). I tested whether queen-rich and queenless colonies accept conspecific queens from other supercolonies/populations and whether the acceptance is related to geographic distance and genetic differentiation (*Fst*) between donor and recipient nests. I tested this in laboratory with 32 queenless and 25 queen-rich colonies originating from five different polydomous colonies (inter-distances 0.22-323 km). I sampled seven different nests from each of the polydomous colonies to create queenless and queen-rich colonies. The queens used in the acceptance experiment were originated from the sampled study nests. The colonies were genotyped with seven microsatellite loci. Queenless colonies accepted introduced alien queens more readily than queen-rich colonies (91% vs. 32%; $P < 0.05$); however, it must be noted that also queen-rich colonies accepted relatively high number of alien queens. The acceptance of alien queens was not related to geographic distance and the degree of population differentiation (P = n.s.). Genetic differentiation had not association with geographic distance (P = n.s.). Accepting unrelated queens into colony may be beneficial because it may increase the genetic diversity of colony. In fact, queenless satellite nests may have an important role in maintaining gene flow between supercolonies/populations.

There's a right time for everything: synchronization of reproduction through policing in a clonal ant

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Relatedness asymmetries due to haplodiploid sex determination are a major cause of intra-colonial conflict among social Hymenoptera. Even though such kind of conflict is not expected in the ant *Cerapachys biroi* because of clonality, attacks toward single individuals are regularly observed within colonies. We used a multidisciplinary approach to understand the causes and the possible adaptive value of these aggressions. *C. biroi*, which is exclusively myrmecophagous, reproduces every 34 days in a cycle of two alternating phases: in the foraging phase larvae are fed brood of other ants until pupation, and in statary phase nymphae complete their development while a new batch of thelytokous eggs is laid by fertile individuals. Our results show that attacked individuals have activated ovaries during the foraging phase, while non-attacked ones activate them exclusively in the statary phase. Moreover, while microsatellite analyses showed that attacked individuals didn't belong to particular or different clonal lineages, they exhibit a specific chemical signature (cuticular hydrocarbons) and quantitatively low cuticular profiles. This, combined with the low melanization levels of their cuticle, suggests that they are young individuals. In *C. biroi* the presence of larvae inhibits ovarian development and thus secures the good functioning of the alternation between statary and foraging phase; attacks are therefore directed toward young individuals that are not sensitive to the larval inhibition of reproduction and thus out of synchronisation with the phasic reproductive cycle of the colony. Policing and executions of those non-synchronized egg-layers avoid perturbations of the alternation of phases. The phasic colony cycle has evolved convergently in many different groups of ants: our study on intra-colonial aggressions in *C. biroi* helps elucidating its adaptive value, and is an important step towards understanding its evolution.

When policing fails: reproductive conflict in *Apis cerana*

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Societies require cooperation between their members to function. In social insects, selfish individuals activate their ovaries and lay eggs, which are reared at the expense of the colony. Thus, worker 'policing', in which workers destroy eggs that were not laid by the queen, has evolved. Reproductive acquiescence theory suggests that in the presence of an effective policing system, the incentive for worker reproduction is removed, and therefore workers should not attempt reproduction. This is supported by studies of the European honeybee *Apis mellifera*, in which over 99% of all worker-laid eggs are removed by police workers and less than 0.1% of workers in queenright colonies have active ovaries. The Asian hive bee *Apis cerana* presents a unique opportunity to test reproductive acquiescence theory. Unlike the majority of honeybees, approximately 6% of workers in *A. cerana* have activated ovaries even when a queen is present. However, in all previous studies, none of the male brood has been found to be worker produced. I present evidence that policing has not been effective in suppressing worker reproduction in *A. cerana*. I regularly sampled workers and male eggs and pupae from four domesticated *A. cerana* colonies. Dissections of the workers showed high levels of ovary activation overall (11.7%). Microsatellite analysis of the male eggs and pupae will determine whether these workers have laid their eggs, and whether any worker-laid eggs were successfully reared to pupation. I also occasionally observed single male pupae developing in an area of worker-sized cells. By transferring worker- and queen-laid eggs into colonies and monitoring their removal rates, I showed that *A. cerana* workers police worker-sized cells less intensively, providing reproductive workers with the opportunity to lay eggs that may escape policing. Thus, the incentive for *A. cerana* workers to reproduce has not been removed by policing pressure.

Assured fitness returns and nest-drifting behaviour in tropical paper wasps

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In eusocial insects, helping in a group may provide a "life insurance" benefit by which the effort helpers invest in the group is assured in the event that they die before the fruits of their efforts are realised (e.g. through maturation of brood). This advantage is called "assured fitness returns". Assured fitness returns models predict that when a helper dies, her investment is preserved because remaining nestmates will raise her partly reared brood to adulthood (Queller 1989, Gadagkar 1990). Brood recycling, slower brood development and increased foraging have been identified as potential mechanisms for compensating the loss of helping effort and thus assuring the investment by dead helpers. Recently, high levels of nest-drifting have been described in *Polistes canadensis*, whereby females have been shown to visit nests other than their own (Sumner et al. 2007). Such individuals seem to act as helpers in the nest they visit and raise the brood of another dominant female to which they are often related. This behaviour may be an effective and immediate strategy to compensate for the loss of individuals via the rapid recruitment of helpers from surrounding nests. We therefore investigated whether assured fitness returns operates in *P. canadensis* and whether nest-drifting is the mechanism to preserve investment of dead helpers. We manipulated the need for help on nests by removing helpers, and monitoring subsequent changes in brood survivorship and helper effort. We found that *P. canadensis* do benefit from assured fitness returns with the investment of dead helpers being preserved through the increased helping effort of remaining nestmates. However, nest-drifting was not the primary mechanism by which fitness is assured. Instead, non-drifting nestmates compensated for the loss of individuals in the nest by increasing their foraging effort (number of trips). Although nest-drifting is widespread in *P. canadensis* and possibly other tropical *Polistes*, ultimate explanations for such behaviour remain unknown.

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A call to arms: directional vibration sensing in the termite *Macrotermes natalensis* (Haviland)

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For a long time it was thought that small insects on solid substrates might be unable to extract directional information from vibratory signals, mainly because time-of-arrival and amplitude difference at different receptors were thought to be too small to be processed. In recent decades several behavioral studies demonstrate the ability of insects to localize the source of vibrations, but very little is known about the cues used for orientation. The termite *Macrotermes natalensis* communicates using vibrational drumming signals transmitted along their subterranean galleries. When soldiers are attacked by predators they tend to drum with their heads against the substrate and create a pulsed vibration. Vulnerable workers respond by a fast retreat into the nests. Soldiers also start to drum, thereby amplifying the intensity of the signal and are recruited to the frontline i.e. the source of the alarm signal.
Here we show that *Macrotermes natalensis* make use of directional vibration sensing in the context of colony defense. Behavioural observations at the termite mount indicate that soldiers make use of vibrational drumming signals as a directional call to arms. In arena experiments on natural nest material soldiers are able to localise the source of vibration. They stay significantly longer at the side facing the source of vibration than on the opposite side. Using two movable platforms, that vibrate termite's legs with different time delays, we can show that difference in the time-of-arrival is the directional cue used for orientation. Time delays short as 0.2 ms are sufficient to be detected. Soldiers show a significant positive tropotaxis to the platform stimulated earlier. To our knowledge this is the first study demonstrating that a difference in time-of-arrival at different legs is the cue used for vibro-tropotaxis in small insects.

Plant selection by leaf-cutting ants: decision-making by foragers and quality control through the symbiotic fungus

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Leaf-cutting ants are polyphagous herbivores that, despite their catholicity, show distinct preferences in the substrate choice for their symbiotic fungus. We investigated to what extent avoidance learning and memory for plant unsuitability for their symbiotic fungus underlie plant selection. We fist tested the acceptability of ten rarely harvested plant species on *Atta colombica* field colonies located in two different habitats, where the tested plant species were either present or not. Colonies in the habitat where the plant species occurred avoided all species on first encounter, suggesting previous experience with them. Colonies without the plant species in their habitat, however, first accepted, but then avoided 4 of them when tested after 24 and 48h. Such an avoidance response towards previously-acceptable leaves could be experimentally induced by infiltrating acceptable leaves with a fungicide not detectable to the ants, but harmful to the symbiotic fungus, indicating that workers learn to reject plants that have detrimental effects on the fungus, but not on themselves. To determine how robust memory for plant unsuitability was, workers from field colonies were offered the previously avoided, yet untreated plant every 2 weeks, and its acceptance monitored in the long term. It took up to 18 weeks until foragers harvested the plant again, indicating the involvement of robust long-term avoidance learning in foragers. We hypothesized that olfactory memory may underlie the delayed avoidance of plants unsuitable for the symbiotic fungus. Laboratory experiments demonstrated that foragers were able to identify the incorporated plants, and to recall information about their suitability for the fungus, through the plant's odour alone. It is argued that the harvesting pattern observed in field leaf-cutting ant colonies largely depends on the workers' foraging experience and their behavioural plasticity, essential in a highly diverse environment where both leaf availability and quality vary throughout the year.

Social learning in bumblebees (*Bombus terrestris*): Just attraction to conspecifics or stimulus enhancement?

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Social learning is an adaptive strategy allowing access to information about the environment from other animals' knowledge instead of relying only on costly personal trial-and-error learning [1]. When deciding from which flower to forage, bumblebees (*Bombus terrestris*) use the presence of conspecifics as a valuable cue to the potential reward status of previously unfamiliar flowers [2-5]. Previous studies suggested that the preference of naive bees for flowers visited by other pollinators could be explained by local enhancement mechanism [6], i.e. simple attraction to conspecifics [2, 3, 7, 8]. But can bees extrapolate to other flowers of the same species, so that by viewing conspecifics on a flower they might instantly prefer flowers with the same properties, without the need to first probe a flower next to a feeding conspecific? In our setup, bees were given a choice between two unknown artificial flower species. A model of a 'demonstrator' bee was presented on half of the flowers of one of the species. We found that bees visited both empty and occupied flowers of the flower species with demonstrators, irrespective of the actual presence of a bee model, while avoiding flowers of the other species. This behaviour indicates *stimulus enhancement* [6], as bees are not simply attracted by conspecifics but by all flowers presenting the same characteristics. In addition, we showed that such social learning behaviour was only triggered if tested bees have past social foraging experience. Thus, social learning in bumblebees is not innate but facilitate by previous positive associations with conspecifics. We also evaluated a potential facility to use conspecific as foraging cue by comparison to non-social cues.

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The foraging success and organisation of polydomous ant colonies

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A polydomous ant colony is one in which the population exists in at least two spatially separated but socially connected nests. The nests function as a cooperative unit and there is exchange of individuals between the nests (Debout et al. 2007). Exchange of food may also take place (eg. McIver 1991; Lanan et al. 2011). We have developed an agent-based model which extends previous models of polydomy by incorporating both recruitment (via pheromone trails and group recruitment) and intracolony transport of resources. The model is first used to investigate the success of having a polydomous colony structure under varying resource distributions. Polydomous colonies discover resources at a higher rate, making them more successful when food is highly dispersed, but their relative success can be lowered by limitations on recruitment success caused by having a dispersed population. Monodomous colonies can have higher foraging efficiency than polydomous colonies by exploiting food more rapidly. We also use the model to reproduce the organisational features of polydomous colonies. Analysis of the spatial structure and the internest trail network structure of a number of published maps of polydomous ant colonies can provide insight into the potential efficiency of polydomous colonies. By assessing the behaviours of agents within our model which produce similar structures we investigate the possible mechanisms by which a colony can effectively maintain and use the internest trail network for distribution of resources. The results provide a number of testable predictions relating to the behaviour of ants within polydomous colonies.

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Collective decision making in honeybees: temperature gradient vs social gradient

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In our experiments we investigated the influence of a social gradient on collective decision making in honeybees. Young honeybees have their preferred temperature at 36°C. Only a minority of single young honeybees find the temperature optimum when they are introduced in a 2-dimensional temperature gradient whereas groups of bees find the optimum very quickly. They are able to distinguish between different qualities of optima (e.g., 36 and 32°C). After these findings we introduced young bees into a thermal gradient and into a social gradient simultaneously. We generated the thermal gradient with heat lamps and the social gradient with immobilized bees in a cage. We placed an empty cage at the optimum as a control. We investigated two different group sizes (6 and 24). In 25% of the trials with groups of 6 bees without immobilized bees in the sub-optimum the bees chose the optimum collectively, in trials without immobilized bees only in 9% of the trials the optimum was chosen. Whereas in 100% of the trials with groups of 24 bees without immobilized bees the bees chose the optimum collectively, in trials with caged bees only in 30% of the trials the optimum was chosen. In 9 % (for 6 bees) and 30% (for 24 bees) of the trials the bees decided to aggregate in the sub-optimum when immobilized bees were present there. In trials without immobilized bees the groups never chose the sub-optimum whereas in trials with immobilized bees the sub-optimum was chosen collectively in 9 % (6 bees) and 30% (24 bees). We could experimentally show that the social gradient influences the aggregation behaviour of young honeybees.

Individual response behavior and collective patterns in bumblebees. How colonies control the temperature of their brood.

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Like many social insects, bumblebees are able to manipulate the climatic conditions inside their nests. This provides considerable independence from environmental conditions and ensures rapid and safe development of the brood. The two thermoregulatory measures employed are incubation (to increase temperature) and wing fanning (to increase evaporative cooling and thereby cooling). These measures allow colonies to maintain their brood at around 30°C, even under strongly fluctuating environmental conditions.
In order to understand how collective patterns like homeostasis emerge, we need a solid understanding of the individual response-behavior underlying these patterns; and of the mechanisms that integrate the responses of the numerous workers of a colony into a functioning unit. Control of nest temperature in bumblebees is a good model system to address these questions.
What is the stimulus eliciting thermoregulative behavior in bumblebee workers? Behavioral observations of laboratory colonies of *Bombus terrestris* showed that brood-age modulates the thermoregulative response: pupae are incubated more than larvae or eggs and are generally 1-2°C warmer that younger brood stages. They undergo less temperature fluctuations; when colonies experience a depletion of their honey reserves, pupae are incubated the longest. Thus, workers invest most heavily into the maintenance of pupal temperatures. Exposing single bumblebees to a heatable brood-dummy showed that workers respond to the temperature of the brood in combination with an olfactory cue present in the brood-wax, which seems to change with brood age.
Workers differ in their response to the stimuli eliciting thermoregulatory brood care behavior. Inter-individual variability is believed to be crucial for the emergence of division of labor in general. I test this hypothesis in experiments with worker groups showing different degrees of inter-individual variability in their fanning response threshold; and show that response thresholds in bumblebees are highly flexible and modulated by the social environment.

Nest enlargement in leaf-cutting ants: relocated brood triggers the excavation of new chambers

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Leaf-cutting ants build their underground nests in an environment that undergoes both daily and seasonal fluctuations of relevant climatic variables. Suitable conditions for the development of the brood and symbiotic fungus may therefore occur at different depths across the soil profile. Ants are therefore expected to track the preferred range of soil conditions, and enlarge their nests accordingly so as to establish new fungus gardens. We investigated whether workers move the brood and fungus to nest locations offering suitable microclimatic conditions, and to what extent the relocated brood triggers the excavation of a new nest chamber. Using laboratory colonies of the leaf-cutting ant *Acromyrmex lundi*, we induced the relocation of part of a colony by lowering the nest temperature, and by offering a better-suited nest site as an alternative. Ants clearly preferred to relocate brood instead of fungus as a response to cold stress. A choice experiment offering two relocation sites, one with brood and the other without brood, clearly showed that any subsequent fungal relocation occurred at a site where brood was present, with most ants aggregating at that site. When given the possibility to excavate at the sites, ants showed a higher digging activity at the site where the brood was deposited. The shape of the structures excavated at the locations with and without brood differed significantly, with more rounded structures excavated around the brood. It is argued that workers preferentially relocate brood to suitable nest places and that the relocated brood spatially guides digging activity and influences the shape of the emerging structure through a self-organized process resulting from the aggregation of workers around the brood.

The fast and the furious: nest-site selection in the giant Asian honey bee (*Apis dorsata*).

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Choosing a new home is one of the most important decisions a honey bee colony is faced with during its life. The quality of the choice made affects the survivability of the future colony, and therefore its reproductive success. For example, the cavity nesting European honeybee (*A. mellifera*) will spend many days searching the surrounding environment for a new home, with individual scout bees looking for cavities which they assess for criteria such as volume, entrance size and moisture levels. Once a consensus on a suitable location is reached the swarm will depart towards their newfound home. There are 11 currently recognized honey bee species, and many of these species have different nesting requirements. These differing requirements can have profound effects on the nest-site selection process. The giant Asian honey bee (*A. dorsata*) migrates seasonally, and produces single comb colonies which hang below large, smooth barked tree branches or rock outcrops. Aggregations of 10s to 100s of colonies on sufficiently large nesting locations are often formed. In this study, I describe the behaviour of *A. dorsata* scout bees as they evaluate a potential nesting site prior to the arrival of a migrating swarm. I also discuss the behaviour of scout bees on the surface of artificially produced swarm clusters. Scout bees repeatedly evaluate old comb fragments prior to swarm arrival, with large numbers of workers arriving in pulses of scouting activity. Bees frequently fight on the old comb surface, suggesting there may be competition between multiple migrating swarms over the same nesting locations. Similarly to *A. mellifera*, *A. dorsata* swarms reach a consensus on a specific location prior to departure, but make a decision in a matter of hours, not days.

Collective decision making of young honeybees in complex thermal environments

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Young honeybees are known from literature to position themselves at 36 ℃ in a linear and rather steep temperature gradient. When introduced in a circular arena with a gradient more similar to the thermal environment that can be found in real brood nests, the majority of single young bees is not able to position itself at its preferred temperature. Interestingly, under the same conditions groups of young bees collectively aggregate at 36 ℃.

We introduced groups of young bees into a complex temperature gradient with a global optimum (36 ℃) and a local optimum (32 ℃) in a ci rcular arena. For generating the gradient we used ceramic heating lamps. In such a gradient groups of bees from 24 bees and above are able to discriminate a local from a global optimum and collectively aggregate at 36℃. Furthermore, groups of 64 bees a re able to react on changes in the thermal environment during runtime by switching off the lamp above the 36 ℃ area. After a cooling phase they rather quickly re-aggregate at the former local optimum of 32 ℃.

We then investigated whether there is a correlation between group size and the time that is needed for making the decision.

Surprisingly we found, that qualitatively there is no difference in the aggregation process of groups of 24 and 64 bees, but a closer look on the time it took those two group sizes to make a clear decision between the two optima shows that groups of 64 bees are significantly faster than groups of 24 bees.

The collective decision making of young bees shows typical characteristics of swarm intelligent behaviour. Besides being robust and flexible, with the density dependent time for decision making it also shows a typical characteristic of such systems.

Division of labour among trunk-trail workers of the harvester ant *Messor wasmanni*

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Division of labour among workers is an important component of the organization of insect societies that largely contributes to their ecological success. Workers tend to specialize on tasks, such as brood tending, foraging or colony defense, often associated with morphological adaptations. The association between phenotype and task is most pronounced in species with morphologically distinct worker castes although some amount of phenotype-task matching is also commonly found in species where workers do not belong to distinct castes but differ in size. In this study we investigate the existence of a size-task matching of the foragers of the ant species *Messor wasmanni* having a monophasic polymorphism. We collected foragers depending on their position on the trails, their movement direction (inbound or outbound) and their behavior. We identified seven categories: 1) workers moving inbound without load; 2) workers moving inbound with load; 3) workers cleaning the trails from no food debris; 4) workers rejecting refuse out of the nest; 5) workers involved in excursion out of the trail; 6) workers cutting seeds directly on a plant; 7) workers moving outbound the nest. For each category we took a sample of 30 workers from 5 distinct colonies spaced by more than 25 meters. For each worker we measured the following body parts: length and width of the head, alitrunk length and width, femur length. Preliminary results suggested the existence of a size-task matching among trunk-trail workers of *M. wasmanni* that could promote an increasing of the efficiency of workers to perform a particular activity outside the nest.

Collective operant conditioning in three *Myrmica* species

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Foragers of three *Myrmica* species, *M. sabuleti, M. ruginodis* and *M. rubra*, were collectively trained to find their food either near pieces of onion (olfactory conditioning) or under a green cube (visual conditioning) according to an operant method of conditioning.
Myrmica sabuleti workers were olfactory conditioned in about 79h, with no latency period; they reached a mean score of 80 – 85%, their performance being higher at early night. When no longer trained, they lost their learning with no latency period, in about 70h, keeping nearly no memory of it. They were visually conditioned, after a latency period, in about 167h; they reached a mean score of 75-80%, their performance being higher during the day. When no longer trained, they lost their learning after a latency period, in about 150h but kept an obvious memory of their learning. Such characteristics are in agreement with the species' navigation system (main use of odors) and visual perception (of poor quality).
Myrmica ruginodis workers could not be olfactory conditioned under normal light intensity. They could be so in darkness but kept no memory of their 'learning'. They slowly acquired visual conditioning, reached a rather high score and presented a strong memory of their learning. These abilities are in accordance with the species' navigation system (main use of visual cues) and visual perception (excellent).
Myrmica rubra workers were soon olfactory conditioned, remembered 10% of their learning and increased their performance in the course of successive conditioning. They were visually conditioned after a latency period, reached a mean score of 80%, remembered 15% of their learning and slightly increased their performance in the course of successive conditioning. Olfactory and visual conditioning becomes so equivalent in the course of time, what agrees with the species' navigation system (similar use of olfactory and visual cues) and visual perception (of middle quality).

Behavioral profile and guiding efficiency of group leaders in the pavement ant *Tetramorium caespitum*

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In the ant species *Tetramorium caespitum*, collective foraging relies on group-mass communication where successful scouts lay a recruitment trail while some of them physically guide a group of nestmates until the food source (Collignon & Detrain, 2010). We conducted experiments to characterize the behavioural profile of group leaders, to understand how these individuals can improve the reaching of the food source and whether they adjust their leading behaviour to the following response of recruits along the foraging journey. Among all foragers having successfully found the food source and starting to recruit workers inside the nest, leaders were individuals that stayed a shorter time in the nest, focused their recruiting activity in the vicinity of the nest entrance and showed a higher rate of contacts with nestmates. We characterized the outward trajectories of leaders from the nest until the food source. We showed that a straight path coupled with a slow walking speed enhanced the probability for followers to reach the food while leaders that walked at higher speed and deviated far from the food-nest beeline were more likely to lose recruits. On their side, leaders did not seem to take care of their audience since they did not reduce their speed or their path sinuosity following the loss of a recruit unlike tandem-running species do (Richardson et al., 2007). Thus, *T. caespitum* leaders are individuals highly active into the mobilization and the transfer of information about food sources to a restricted number of recruits. However, unlike tandem leaders, they are behaving as leaders without their knowing since they did not exert any control on the following response of their audience.

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Short-term effects of changing food conditions on the polydomous nest-network of *Formica lugubris* in England.

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The red wood ant *Formica lugubris* is polydomous in England (Gyllenstrand & Seppä 2003), but it is unknown if or how they use this network to exploit their environment. Previous work on red wood ants has shown that individual ants have a high route fidelity, with many ants staying loyal to a particular foraging trail, even after winter quiescence (Rosengren & Fortelius 1986). However it has also been shown that, as a whole, the colony is able to respond to changing food conditions, even matching the ideal free distribution (Lamb & Ollason 1994).This has only been investigated in monodomous colonies: in this study we are investigating if and how red wood ants use their polydomous network to take advantage of changing food sources in the environment. This will give valuable information on how this keystone species interacts with the forest ecosystem.

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The evolution of division of labour in primitive and advanced ants.

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The evolution of eusociality is a heavily researched area, but relatively little research effort has been applied to the transition from simple eusocial societies to complex ones. This transition, characterised by increasing division of labour, both reproductive and non-reproductive, has been the subject of theoretical models, both verbal and mathematical, but the predictions of these models have rarely been tested empirically [1]. Many of these models have been based on the observation that social complexity appears to be associated with colony size, an observation which culminated with the recently formalised size-complexity hypothesis [2]. This suggests that as colony size and division of labour are positively correlated due to the reduced chances of any given worker gaining direct fitness in larger colonies [3]. This leads to the prediction that species with larger colony sizes should show greater queen-worker dimorphism (reproductive division of labour) and greater division of labour within the workforce. Here I present a large dataset of social, ecological and morphological data for the Attini, a neotropical tribe of fungus-growing ants, and the Poneroids, a diverse grouping of “primitive” ant subfamilies, and phylogenetic trees for these groups constructed using supertree and supermatrix methods. Whilst controlling for phylogenetic and spatial autocorrelation, these data formed the basis of a comparative study to investigate a correlation between measures of division of labour and colony size.

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How *Myrmica rubra* foragers find their way while foraging?

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After having analyzed the navigation system of *Myrmica sabuleti* and *M. ruginodis*, the eye's morphology and the visual perception of *M. sabuleti, M. ruginodis* and *M. rubra*, we could not but examine the navigation system of *M. rubra*. To do so, we differentially olfactory as well as visually conditioned ants of six experimental colonies and tested then them in mazes judiciously provided with the learned cues. Our results were as follows:
Mymica rubra foragers could find their way through mazes provided with:
-olfactory cues, doing so better under low light intensity,
-visual cues, doing so better under high light intensity,
-correct visual and olfactory cues under any light intensity,
-wrong visual cues and correct olfactory ones, under low light intensity and in nearly darkness.
They wrongly moved through mazes provided with:
-correct visual cues and wrong olfactory ones, under low light intensity and in nearly darkness.
They could not find their way through mazes provided with:
-wrong visual cues and correct olfactory ones, under high light intensity.
These ants thus use olfactory as well as visual cues to navigate, relying more on one kind of cue when the other one is less available.
This navigation system is in accordance with the species' visual perception (of middle quality) as well as olfactory and visual learning abilities (equivalent, in the course of time).

Ant activity and temperature regime in nests of wood ants *Formica polyctena*

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Repeated temperature measurement was carried out in twelve nests of wood ants *F.polyctena* situated in Šumava Mountains in Czech Republic. Nest temperature in different depths was sampled five times a day; simultaneously environmental variables were recorded. Results revealed high variability of inner nest temperature especially in surface layers. Inner nest temperature in period of ant activity (April – August) exceeded 20^{o}C, with highest temperatures reached in June while September temperatures were the lowest in whole sampled period. This confirm that nest heating is timed to assure best conditions for egg laying and brood development in early summer. Temperature difference between the mound depths 10 – 5 cm was in average positive, negative values counted for 10% from all cases. Positive difference indicates that the heat flows from inside out, suggesting high importance of inner heat sources. Similar daily temperature regime occurred in most nests: The lowest temperatures were observed one hour before sunrise, during day inner nest temperatures were slowly increasing till the evening maximum. Surprisingly the temperature didn't peak at midday when the highest air temperature and biggest solar income occurred. The delay in nest heating might be caused by excellent isolative properties of nest material, which slow down the heat transfer. Daily temperature regime is supposed to correlate with foragers' activity and the external heat brought into the nest by ant bodies. Ant activity, counted as number of forages on the trail and number of nest openings, is strongly affected by surface temperature, which depends on air temperature and sun radiation income. The higher the surface temperature the more ants going out of nest. Number of nest openings corresponds with nest temperature negatively whilst increased moisture supports nest opening. This indicates that ants may trade off temperature for humidity.

The coexistence of the plesiobiotically associated colonies of *Formica fusca* and *Camponotus vagus*

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The plesiobiotic association is the most rudimentary form of social symbiosis (Hölldobler & Wilson, 1990) among ant species, which means the regular and casual nesting in close vicinity of different species. The plesiobiotic partners are differ from each other behaviourally, morphologically, they belong to different genera (Hölldobler & Wilson 1990) and they have different competitive ability, as a rule. The different colonies do not mix, and they are potentially hostile to each other. Recent studies have revealed that *Formica fusca* (Linnaeus, 1758) co-occur with other species in plesiobiotic association more frequently than expected by random (Czechowski 2004, 2005; Włodarzczyk et al. 2009).
We also found plesiobiotic relationship between *F. fusca* and *Camponotus vagus* (Scopoli, 1763). The main addressed question of our study was, which factors make possible the coexistence of the two species. We studied both species' colonies in laboratory and in field. In the laboratory experiments the relation between the two species was studied in smaller foraging range and we tested food type dependency, too. The field observations were carried out in two forests, with and without *C. vagus*. Ant nests were mapped in randomly selected plots at least 100 m^2. We measured the distance between nests and we tested the aggression between workers from neighbouring and distant colonies. We used baits to observe the frequency of coincidence of different species' workers.
In laboratory arena we observed exclusions of *F. fusca* by *C. vagus* from the food sources. There were significant differences between the two species' colonies in connection with food type and distance dependency. In the mapped plots we found that the average distance between *C. vagus* and *F. fusca* nests was significantly smaller than the average distance between conspecific *F. fusca* nests, assuming that *F. fusca* may have benefits from the vicinity of the superior competitor *Camponotus*.

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Slavemaker select for high aggression in host colonies

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The obligate slavemaking ant *Protomognathus americanus* reduces the fitness of *Temnothorax longispinosus* host colonies in their vicinity through reoccurring slave raids. During a single raiding season, slavemakers can attack up to ten neighboring colonies, drive away or kill the adults and steal their brood. These destructive raids often result in the death of the host colony. As this host-parasite interaction is dominated by aggressive behavior, it has always been assumed that host aggression should be beneficial. However, this assumption has never been formally tested. In this study, we investigated if more aggressive colonies are better in defending themselves and their brood against slavemaker raiding attacks. We measured the aggression of host colonies against non-nestmate conspecifics in a standardized assay. Thereafter, we confronted each host colony with a slavemaking colony. The entire raiding process was recorded and the host's success in nest defense was estimated as proportion of the brood they were able to evacuate. Our results showed that colonies which behaved more aggressive against intruders in a standardized essay also rescued a higher proportion of brood. However, more aggressive colonies paid a price for their increased defensiveness as they suffered from higher worker mortality. As aggression on the colony level has been found to be consistent over time and the emergence of a new worker generation, it is presumably largely genetically determined. Hence, we would expect strong selection on high aggression, which should result in the presence of highly aggressive host colonies in areas in which the slavemaker is present. We find pronounced variation in host aggression in the field, which indicates that this behavioral trait could be associated with costs. The association of increased host aggression and worker mortality could present such a cost, counter-balancing the benefits of aggression.

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The "SOS" in *Cataglyphis cursor* ants

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Rescue behaviour in non-human animals has been observed occasionally in the wild and recently has been experimentally explored in *Cataglyphis cursor* ants (Nowbahari et al. 2009). In *C. cursor* species, as in some other insect societies, young ants less than 4 days after emergence are able to be adopted in foreign colonies whereas adult ants never are adopted and are attacked instead (Nowbahari & Lenoir 1989). The period shortly after emergence is critical for physiological maturation. Nonetheless, it is not known if workers less than 4 days old already carry the colonial odours and if they are able to call for or receive help when trapped. To explore our understanding of the signals triggering rescue behaviour and its ontogenesis, we conducted both behavioural and biochemical analyses of experiments in which mature rescuers were tested with ensnared workers of different age categories, namely newly hatched ants under 3 days old (callows), young ants that were 10 days old, and mature adults. Control tests were conducted with same-age victims made dead by chilling. Volatiles released by victims in each of the three live-victim groups were subject to SPME (Solid Phase Micro Extraction) chromatography. Our results show that rescue behaviour was directed toward all three types of live victims. The biochemical analysis revealed the presence of volatile components in both ten-day-old and mature workers, but not in callows. The chemical signal appeared to be highly similar to alarm signals found in *Formicinae* ants. Rescue behaviour elicited by 10-day-old and mature ants likely was triggered by some of the molecules emitted by victims, whereas rescue behaviour elicited by callows probably depended upon their strong attractiveness.

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Cataglyphis cursor ants are able to discriminate between individual nestmates

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In social insects, recognition of nestmates allows social cohesion and is key to understanding how altruistic behaviours benefit close kin. Recognition is thought to be based on a "gestalt" model, wherein individuals mix their odours into a unified chemical colonial label (Crozier & Dix, 1979). Nonetheless, individual recognition is advantageous whenever dominance hierarchies control the partitioning of work and reproduction (Hölldobler & Wilson, 1990). Individual recognition is known to exist between heterocolonial individuals (Nowbahari, 2007) and between unrelated founding queens of the ant *Pachychondyla villosa* (D'Ettorre & Heinze, 2005). Here, using an habituation-discrimination method, we show that, in the monogynous and polyandrous ant *Cataglyphis cursor*, workers are able to discriminate individually between closely related nestmates. After a familiarisation process, ants spend more time investigating non-familiar than familiar sisters in a discrimination task. We hypothesize the existence of "preference networks" whenever multiple mating of queens (i.e., polyandry) (Pearcy et al. 2009) results in individual odour differences between sisters and half sisters. These results expand our understanding of social insects' cognitive capabilities and suggest that a more dynamic system of interactions, in which individual preferences play an important role, may be present in many insect societies.

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Interindividual variability in sucrose response thresholds and division of labor in the ant *Camponotus aethiops*

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Division of labor, in which individuals specialize in particular tasks, such as foraging or brood care, is the hallmark of insect societies and contributes to their ecological success. The mechanisms underlying division of labor have been studied in the honeybee by focusing on interindividual variations in sucrose responsiveness, using the proboscis extension reflex (PER) of restrained bees. By stimulating the antennae with increasing concentrations of sucrose solution it was possible to show an individual variation in the response to the stimulus, and this variation correlates with the task performed by the bees. Thus, the current model explaining regulation of division of labor in social insect is the "response threshold model" based on individual variability in response to biologically relevant stimuli. We investigated individual variability in sucrose response thresholds in the ant *Camponotus aethiops* using the maxilla-labium extension reflex (MaLER), an appetitive response comparable to the PER of bees, and that allows measuring response thresholds in restrained ants whose antennae are stimulated with increasing concentrations of sucrose solution. We show that individual ants performing different behavioral tasks (foragers, nurses or "inactive") have different sucrose response thresholds. Foragers respond to water and low sucrose concentration (low response threshold), which allows water transport to the colony and forage on all the potential sucrose sources, including extrafloral nectar. Inactive workers respond only to the highest sucrose concentration (high response threshold), which is certainly related to the fact that they store nectar in their abdomen in case of sucrose shortage and they should thus only store the most valuable sucrose solutions (highest calorie diet). Nurses, which take care of the brood, respond to intermediate sucrose concentration (intermediate thresholds); they are in principle younger than foragers and inactive workers, so that they can modulate their response threshold and specialize in different behavioral tasks as age progresses.

Facial marking as quality signal in the hover wasp *Liostenogaster vechti* (Hymenoptera, Stenogastrinae)

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Both recognition and conventional signals are surprisingly common in the animal kingdom. Even if invertebrates, and in particular social insects, primarily use chemical cues for communication they are also able to use the visual channel. In the last decade both observational and experimental evidences have shown the existence of both quality signals and individual recognition cues in some species of Polistes paper wasps. However these findings, the only available for insects, remain a source of much controversy. Here we show that at least one species of hover wasps (Hymenoptera, Stenogastrinae) use the visual channel for social communication. We demonstrate using morphological and anatomical measurements and behavioural assays that the size of dark facial marking of females of *Liostenogaster vechti* are related to reproductive dominance and represents a badge of status. Our results reveal that visual communication is probably more widespread among social insects than previously thought.

Brood presence and available space influence digging activity and lead to nest-size adjustment in leaf-cutting ants

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In ant colonies living underground, the nest size positively correlates with their worker number, which suggests that workers may respond to changes in available nest space occurring during colony growth by enlarging the existing structure. We investigated whether variables other than the worker number influence the excavation activity, and to what extent ants adjust the size of their nests. To examine this, we allowed workers of laboratory colonies of *Acromyrmex lundi* to dig in clay arenas that offered either reduced or ample space, with presence or absence of brood, which is known to attract workers to the site. In independent series, the brood was offered in increasing numbers. Workers confronted with reduced space excavated more than workers encountering ample space, although their number was the same in both setups. Even more space was excavated when ants encountered a reduced space and brood was present, although digging activity did not increase with increasing brood numbers. When ants encountered ample space, the presence of brood did not influence digging activity. Ants were observed to adjust the nest size by depositing soil pellets in previously-excavated areas, thus reducing the available nest space. The final nest size was similar in ant groups that initially had either a reduced or an ample space, except for the group with reduced space and brood present, which had a larger final nest size and deposited less pellets inside the excavated structure. Ants reacted to the presence of brood by excavating a larger chamber area, and showed no marked reduction of chamber space through pellet deposit. Our results indicate that the final nest size is not simply a direct function of a colonies workforce, but that workers' digging activity is flexible, with workers responding to the available nest space and presence of brood.

Extremely male-biased sex-ratio in a socially polymorphic sweat bee (*Halictus rubicundus*)

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Many ants, termites, bees and wasps live in complex societies, where most individuals forgo their own reproduction and help raise offspring of queens. To explain this worker behavior, the subfertility hypothesis postulates that if there were individual differences in fecundity, the best option for an individual with bad breeding prospects might be to forfeit its own reproduction altogether and instead help kin. We set out to test experimentally the subfertility hypothesis in the primitively eusocial sweat bee *Halictus rubicundus*. Nest foundresses provision eggs with food and then seal the brood chambers. Since fecundity is dependent on body size (larval food provisions) in insects, we aimed to get bees of different sizes in the nests by introducing different sized pupae from the wild. Foundresses successfully bred in the lab. However, none of the offspring introduced from wild nests choose to work. Additionally, the foundresses themselves surprisingly produced heavily male biased broods. Even compared to a wild asocial population, the male bias was extreme. We suggest that elevated temperature experienced during this spring might have caused this male bias. This raises interesting questions regarding the resilience of these bees towards the warming climate.

Antennal drumming in the primitive eusocial wasp *Mischocyttarus (Megacanthopus) parallelogrammus* (Hymenoptera, Vespidae)

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Social wasps have a well-developed food interaction between adults and their offspring with a twist: the larvae, as well as being fed, produce salivary secretion that is imbibed by the adults [1]. Moreover, in social wasps of independent foundation, contact with the larvae is accompanied by remarkable mechanical signs of communication [2]. Colonies of *Mischocyttarus parallelogrammus* were observed weekly in the Fazenda Angelim Rainforest, Ubatuba, São Paulo, Brazil. During the feeding context, it was observed that adults performed the antennal drumming when returning with preys to the colony. After macerating the prey, the adult checks the cells, feeds the larvae and then quickly hit their antennae on the interior walls of the cells. With the use of a professional directional microphone, sonograms were obtained, demonstrating the occurrence of vibrations caused by the beating of the antennae in the nest at a mean of 13 ± 2.56 beats per second. Such a vigorous behavior that expends a high rate of energy and was observed only in cells containing larvae as from third instar when the larval saliva production becomes significant, suggests an at least relevant function. This behavior could be related with the offering of the resulting liquid from macerated prey stored in the crop of the adult to the larvae and this would have the function of inhibiting the release of larval saliva [3]. However, it could also be a signal to the larvae to release larval saliva to adult [4]. The first hypothesis is the most well defended as some studies have found that the flow of food is unidirectional and always from the adult to the larvae [3]. However, this behavioral act is still much debated, but it is known that this is a communication between adults and immature of social wasps, and had not yet been described for the genus *Mischocyttarus*.

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The effect of social isolation on physiology, behavior and fitness in ants

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Social animals are exposed to the social communication at every moment. Individuals are affected by some stimulation emitted by other members of group. At the same time, separation from other members of group leads to the modification of physiology and behaviors, finally affecting their fitness. In social insects, individuals belong to respective castes and live in the societies. Because of their strong social bonding, they usually cannot survive in complete isolation. However, the mechanisms involved are still unrevealed. We focused on the effect of social deprivation in ants *Camponotus fellah*, and try to find out which kinds of social interaction functions to keep ant survival, and how their physiology or behaviors are modified using ant-tracking system. Isolated ants showed striking shorter life span compared with grouped condition in which they are kept with 10 nestmates. Old worker ants are more susceptible to isolation stress than young ones. Additionally, we found that not only the grouped-condition, but also the interaction with brood has effects to recover the survivorship of isolated ants. Ant-tracking system provided the evidence that their behavior, and the levels of activity are different between isolated and grouped ants, and also between dead and survived ones. Based on the survival analysis and behavior-tracking, I want to discuss about the process how social interaction functions to keep the fitness in ants.

Articulate communication with an unintelligible vocabulary

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The way that cooperating individuals within a group share information is expected to reflect in their collective performance. We study the rudimentary recruitment behavior of the desert running ant, *Cataglyphis niger* which is facilitated by contact-dependent pairwise interactions. Estimating the information capacity of these interactions reveals that the vocabulary available for this process is small, less than two "words". This disability to differentiate between messages makes the system highly susceptible to activation by random noise. We show how, using simple rules, the ants manage to carry out their collective task despite their limited communication skills. Finally, we demonstrate surprising group level, density dependent manifestations of these single-ant behavioral rules.

When worlds collide

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Honeybees, wasps, ants and termites are special among social insects since their nests function as centres for information transfer and recruitment. Recruiting to a new food location, can be achieved by a knowledgeable individual informing the colony the precise location of the food and/or by bringing back a sample of food to the nest. While most studies have researched the effect of the environment outside the nest on the type of information transferred, just a few have studied the characteristics of information transfer resulting from the environment within the colony. The model of Dechaume-Moncharmont et al., 2005, predicts that it is more beneficial for ants within a colony to search for the new food source, of which they have been informed through food samples from returning foragers, than to wait to be informed on its location. However, when starvation levels are high the model predicts that it is more beneficial for the ants to be recruited to the precise location. We investigated the type of information transferred by tandem running ants, *Temnothorax albipennis* when the colony was fed and starved. Following the model's prediction, our results indicate that tandem runs, i.e. information on the exact location of food, occur only when the colony is starved. Interestingly, tandem runs lasted only for a short window of time. The results might suggest that the foraging begins with an immediate need for a relief from starvation, which is achieved by introducing only food to the colony as opposed to information on the location of food. After this phase slow and accurate tandem runs increase the number of knowledgeable foragers until there is sufficient number of knowledgeable foragers for the colony's recovery. Thus, our study emphasizes the importance of the environment within the colony to understand social insects foraging.

Dechaume- Moncharmont et al. 2005 Proceedings of the royal society B 272:1689-1695.

Individual reproductive benefits shape labour division in the ant *Platythyrea punctata* and the ant *Pseudomyrmex gracilis*

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Eusocial insect colonies are characterised by labour division among reproductives, nurses and foragers. Age polyethism, *i.e.* division on young nurses and old foragers, is the most prominent proposed mechanism. Several studies, however, have documented deviations from this simple pattern, suggesting that the evolution of labour division may be affected by additional genetic, social, and environmental factors in manifold ways.
Here we documented that in the parthenogenetic ant *Platythyrea punctata*, where all individuals in a colony are assumed to be totipotent and potentially able to reproduce, foragers separated from reproductive individual usually do not reproduce contrary to nurses which all start to reproduce after separation. In few cases of forager reproduction, foragers always started to reproduce at least two weeks later than control nurses and laid on average 5 times less eggs. Reduced fecundity of foragers was irrespective to individual age, life expectancy and foraging experience, *e.g.* ten-day-old naïve individuals collected on their first foraging trip had already decreased fecundity in comparison to nurses of the same age.
We observed similar division into less potentially fecund foragers and high potentially fecund nurses in the elongated twig ant *Pseudomyrmex gracilis*. Though the species has distinct queen and worker castes 22% colonies collected in the field were queenless and consisted egg-laying workers.
The results show that in species where individual reproduction is not a rare event, due to colony splitting (*P. punctata*) or death of reproductive (*P. gracilis*), labour division based on differences in potential fecundity is predominant over the division based on age, life expectancy or individual experience. As so far proposed mechanisms explaining evolution of labour division focused more on increasing overall colony performance here we show that, at least in some species, individual perspective, *i.e.* benefits from direct reproduction, may also strongly shape division into foragers and nurses.

Session 2. Mating systems, dispersion and population structure

Males do not like the working class: evidence of male choice in a social insect

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In the last decades an ever-growing body of evidence has undermined the sexual selection stereotypes of female “choosiness” versus male “undiscriminating eagerness” to mate crystallized in the Bateman’s principle. Despite being demonstrated in a soaring number of both vertebrates and invertebrates species, male mate choice in social insects has never received much attention. In the female-centered hymenopteran societies males have often been regarded as merely short-lived "flying sperm containers" or “simple and small mating machines”, spending almost all their time and energies in trying to achieve a mate. They have to cope with an ephemeral lifespan, a limited not refillable set of sperm, and, often, with the constraint of being physically allowed to mate only once or few times in a lifetime. Finally, they usually encounter females belonging to different castes, which dramatically differ in their reproductive potential. Hence, without the ability of discriminating reproductive partners from non-reproductive females, males may waste their already scarce chances of reproduction. Here, we report the first evidence of male mate choice based on female castes in the primitively eusocial paper wasp *Polistes dominulus*. By presenting males with females of different reproductive potential and recording the number of sexual interactions we showed that males exert a mate choice based on female caste, strongly preferring gynes (i.e. future queens) with respect to workers regardless of female age, body size or health condition. Our study offers a first proof that social hymenopteran males are not always “small mating machine” eager to mate and competing to be chosen, sometimes they also get the chance to choose. This finding sheds new light on the study of male phenotype in the female-dominated hymenopteran societies.

Mating-induced changes in the behaviour of *Leptothorax gredleri* ant queens

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Most organisms undergo behavioural changes throughout their lives. These behavioural changes are often necessary and may be a response to environmental changes. They have been exemplified in taxa as diverse as birds, fishes, etc. for activities like mating or migration. Social insects are very convenient models for studying behavioural changes. It is well known, for example, that workers in most social insect colonies shift from one task to another as they become older. Reproductive female ants go also through strong behavioural transition. At their earliest life stage, young female sexuals are cared by workers in their maternal nests. Later, male and female sexuals mate in a nuptial flight. After mating, young queens start a new colony by digging a nest and laying eggs. Thus, they experiment, on a very short period of their life, dramatic changes both at behavioural and environmental levels. Unlike other ant species, *Leptothorax gredleri* Mayr, 1855 female sexuals exhibit a "female calling syndrome": virgin female sexuals climb on grass stems and attract males with sexual pheromones. Once mated, it has been suggested that these young queens may seek readoption in their maternal nest. Although opportunistic observations were reported, there have been few data to date examining and quantifying behavioural changes of ant queens. We therefore investigated under laboratory conditions the mating-induced changes in the behaviour of *Leptothorax gredleri* ant queens. To this end, we tested the phototactic, geotactic and locomotory behaviours of *L. gredleri* ant queens at five different stages of their life. Nest site selection and readoption were also conducted under artificial conditions. Our results showed that female reproductives exhibit different behavioural phases before and after mating. These behaviours parallel the biological life cycle of these females.

Genetic polyethism in the polyandrous desert ant *Cataglyphis cursor*

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Several genetic and non-genetic hypotheses have been formulated to account for the evolution and maintenance of multiple mating by females (polyandry) in social Hymenoptera. A major hypothesis argues that polyandry allows production of a genetically diverse worker force, enhancing division of labor and colony task efficiency. We tested the relationship between patriline, worker size and task specialization in the desert ant *Cataglyphis cursor*, a species showing natural variation in queen mating frequency. Our results show a significant association between patriline and task preference: workers belonging to different patrilines differ in their propensity to perform a given task (foraging, nest construction, waste management or food-storage). Furthermore, we found that worker size is closely associated with task specialization, but not with paternal origin. Overall, these results indicate that division of labor is at least partly genetically influenced in the ant *Cataglyphis cursor*, lending support to the 'polyandry for a more efficient polyethism' hypothesis

Loss of flight in insects: the special case of ergatoid queens in ants

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Many solitary insects no longer fly, often because a loss of costly wing muscles leads to increased fecundity. These wingless or short-winged forms exhibit various degrees of simplification in thorax morphology. Among flightless insects, ant workers have some of the more extremely reduced thoraces, reaching complete fusion of all sclerites. Workers in many species are also much smaller than conspecific winged queens, further highlighting their distinctness as a less-costly phenotype. Even though flight is restricted in ants to a brief dispersal event, winged queens have been selected against in many independent lineages. Thousands of species have wingless (or short-winged) reproductives that occur together or instead of winged queens (Peeters 2012). "Ergatoid queens" broadly describes any reproductives (with a functional spermatheca) lacking wings but morphologically different from workers. Comparative study reveals great structural heterogeneity, which is not surprising given the multiple evolutionary origins. According to species, ergatoid queens can be more similar to either workers or winged relatives. Importantly, fecundity is variable as in species with winged queens and it is not affected by flightlessness, presumably because of colony-level selection. Ergatoid and brachypterous queens appear to be novel phenotypes that evolved by uncoupling the growth of thorax and abdomen. Their mosaic nature indicates that both worker- and winged queen-specific morphological modules became recombined during evolution (Molet et al. 2012). Unlike flightless insects that live and reproduce alone, flightless reproductives in most ant species have lost the ability to start new colonies alone, and they completely rely on nestmate workers during dependent colony founding. Many social insects show colony-level adaptations for dependent colony founding, but ants also exploited worker-specific innovations to evolve cheaper flightless reproductives.

Peeters C. 2012. Myrmecol. News 16: 75-91
Molet M., Wheeler D., Peeters C. 2012. Am. Nat. in press

Small and large scale implications of independent colony foundation in the European common black ant, *Formica fusca.*

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The mode of colony foundation in ants has implications for the individual queen and the nests she founds, but also for larger spatial processes such as sex-biased dispersal and population substructuring. Using a combination of mitochondrial and nuclear (microsatellite) molecular data we explore the genetic patterns and consequences of independent colony foundation in the European common black ant, *Formica fusca*. Two populations (~5 and ~11 km ^{2}) were exhaustively sampled (small scale dataset) and queen composition (number of queens, number of mitochondrial haplotypes and relatedness) in polygynous nests, and patterns of worker nestmate relatedness in both polygynous and monogynous nests were estimated, and the relationships between these were tested with GLMs, accounting for nest size and population. Another 12 populations were sampled for workers on a large scale (ca 50x10km) and typed with the same molecular markers. Neighbourjoining trees (mtDNA), genetic differentiation (F_{ST}; mtDNA and microsatellite data), network analysis and principal component analyses (microsatellite data) were used to investigate patterns of dispersal (males vs. queens), gene flow and spatial substructure. Mitochondrial DNA diversity was high on both spatial scales, with several haplotypes found in a single patch, as well as in a minimum of 10% of the polygynous nests. On the small scale nestmate queen relatedness increased significantly with the number of queens in the nest, but nestmate queen relatedness patterns were independent to nestmate worker relatednesses. On the large scale both types of molecular marker suggested panmixia, indicating very high dispersal in both queens and males. We place our results in the context of *Formica fusca* as a pioneering species with large effective population sizes, high dispersal capacity and rapid queen turnover.

Cryptic structure of native ant supercolonies

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According to the inclusive fitness theory, some degree of positive relatedness is required for the evolution and maintenance of altruisms [1, 2]. However, ant colonies are sometimes supercolonial, i.e. networks of interconnected nests spanning over large areas. These are typically genetically homogenous entities with zero or low nestmate relatedness, causing a putative problem for the theory [3]. We studied spatial structure and genetic relatedness in two supercolonies of the ant *Formica exsecta*, by using nuclear and mitochondrial markers. We show that there may be multiple pathways to supercolonial social organization, leading to different spatial genetic structures. One leads to a largely homogenous genetic structure across the population, as expected if supercolonies are founded by a small number of colonizers, followed by nest propagation by budding and domination of the habitat patch. The other pathway leads to a genetic mosaic of nuclear and mitochondrial clusters, which is difficult to explain with our current knowledge about the integrity of ant colonies and their nestmate recognition systems. A mosaic genetic structure could arise from immigration or sexuals dispersing by flight within the supercolony, but it would need atypically strong and continuous migration rate. Rather, simulation of a stepping-stone model shows that discontinuous nuclear clusters can arise from random but restricted movement of individuals. Finally, amalgamation of nests in an originally multicolonial population is a potential but controversial possibility for the discontinuous distribution of mitochondrial haplotypes. Relatedness among nestmates was low in both populations, but increased significantly when estimated among individuals sharing the same nuclear cluster. However, increased relatedness has no biological meaning, if cooperating and competing individuals are mixed in the population.

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Is ejaculate quality in *Apis mellifera* drones affected by senescence, food provisioning and/or immune challenge?

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Spermatogenesis in eusocial insect males has ceased when they eclose from the pupal stage so that sperm stores can never be replenished. Males will die shortly after mating, whereas queens often live for years, relying solely on the sperm received during a single mating event early in life. High sperm quality is therefore essential to male reproductive success and selection should have produced sperm-quality-stabilizing mechanisms as males cannot predict on what day of their short life they will copulate. An ejaculate consists of both sperm and seminal fluid, which contains many proteins that likely affect male reproductive potential, both before and shortly after ejaculation when sperm competes for access to the queen spermatheca. I used honey bee drones (*Apis mellifera*) to test: 1) whether ejaculate viability decreases across the lifetime of drones, 2) whether depriving males of protein in their diet compromises ejaculate quality, and 3) whether ejaculate viability is affected by a mild immune challenge. I used live/dead fluorescent staining techniques and fluorescence microscopy to measure sperm viability in ejaculates, and found that sperm viability decreased with age in some colonies, but remained constantly high in others, raising interesting questions about the apparently variable mechanisms for avoiding ejaculate senescence after hatching. Diet protein content had no effect on sperm viability, regardless of whether the restriction was initiated immediately after hatching or after drones had become sexually mature. This suggests that males have all necessary resources for producing the best possible seminal fluid by the time they eclose. However, a mild immune challenge, via puncturing the intersegmental membrane between the tergites with a syringe, negatively affected sperm viability, suggesting that some resources that maintain ejaculate quality can be allocated to other functions when drone survival before mating is at stake.

Waste of gametes: Hybridization in the face of strong selection

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Hybridization can be a major force in speciation and create evolutionary novelties. Genetic compatibility problems between hybridizing genomes usually lead to transmission ratio distortion (TRD) and in vertebrates can even trigger clonality and elevated ploidy levels. Our previous study found a unique outcome of hybridization in a population most likely derived from hybridization between wood ants *Formica aquilonia* and *F. polyctena* (Kulmuni et al. 2010). The population consists of two genetically differentiated groups, where previous hybridization and gene flow has resulted in sex-specific TRD. This leads to stable genetic differences between males and females. We proposed two possible mechanisms that could lead to such sex differences. They could be caused either by strong postzygotic selection eliminating incompatible genotype combinations or by prezygotic segregation separating paternally and maternally inherited chromosome sets into male-forming and female-forming gametes. Here we test these hypotheses. Laboratory cultured colonies show that queens lay eggs recombining the maternal and paternal alleles and produce all genotype combinations thus rejecting the segregation hypothesis. Rather, both diploid and haploid individuals demonstrate that many genotypes observed in eggs disappear or are drastically reduced in frequency in later developmental stages (larvae, pupae, adults). This suggests that selection eliminates these genotypes at egg or larval stage and creates differentiation between sexes wasting a lot of gametes in the process. Our results are in contrast to the clonal reproduction that has been shown to underlie differentiation between males and females in other species of ants. Similarly, in vertebrates differentiated genomes are transmitted by some form of clonality. The hybrid population studied here has retained sexual reproduction even in the face of extreme mortality.

Kulmuni J., Seifert B., Pamilo P. (2010) Segregation distortion causes large-scale differences between male and female genomes in hybrid ants. Proc Natl Acad Sci USA. 107(16): 7371-7376.

Clonal reproduction and invasiveness in the longhorn crazy ant *Paratrechina longicornis* (Latreille)

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The crazy ant Paratrechina longicornis is a highly invasive tramp species present in virtually all tropical ecosystems. Recent genetic analyses of an introduced population (Bangkok, Thailand) of the crazy ant revealed that queens and males are clonally-produced, whereas workers develop from fertilized eggs (Pearcy *et al*., 2011). Clonal reproduction of sexuals results in separation of female and male gene pools, allowing new queens to mate with their nestmate "brothers" without incurring the costs of inbreeding. For this reason, this remarkable reproductive strategy should allow foundresses and their offspring to circumvent the costs associated with limited population size and bottlenecks and, hence, facilitate the settlement of introduced populations in remote locations and isolated areas. Consistent with our previous results, recent genetic analyses of workers from other introduced populations (USA, Venezuela, Spain, Cameroon, Indonesia and Taiwan) revealed that clonal reproduction for both sexes was characteristic of all *P. longicornis* populations sampled. Only one queen and one male genotype were found in most populations, showing that they may actually derive from a single-queen introduction. Similar introduction patterns have been described for the little fire ant *Wasmannia auropunctata* (Mikheyev *et al*., 2009), where queens and males are usually clonally-produced in introduced populations. However, in contrast with the data published for *W. auropunctata* (Foucaud *et al*., 2009), genetic analyses suggest that all introduced populations sampled so far derive from invasion by one single and extremely successful queen and male lineage. In conclusion, the presence of only one queen and male lineage in most populations strongly supports the hypothesis that this mode of reproduction is an important pre-adaptation explaining the tremendous invasive success of the species.

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Chemical-physical soil properties of the nesting sites of two syntopic harvester ants: *Messor wasmanni* and *Messor minor*

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The distribution and abundance of ground-nesting ants could be influenced by biotic and abiotic factors. Concerning abiotic factors, some of the chemical-physical properties of the soil (i.e. pH, moisture and texture) may affect the most vulnerable stage of colonies life-cycle, colony founding, influencing the micro- and macro-distribution of adult colonies. It is also known that ground-nesting ants can directly modify the microhabitat characteristics of nesting site due to workers activity.
The seed-harvester ants *Messor wasmanni* and *Messor minor* are commonly present in Mediterranean grassland ecosystems of Central-Southern Italy. The two species show a similar ecological niche concerning several aspects of their life-history traits and a broad overlapped distribution of their nests.
In the present study we investigate the effect of different abiotic factors, moisture and soil texture, on the survival rate of newly-mated queens of *M. wasmanni* and *M. minor* by means of three-factors laboratory experiment (2 ant species x 3 texture soil types x 4 moisture levels). Samples were collected after September nuptial flights in a typical grassland area of the Estate of Castelporziano, a Natural Reserve near Rome (Italy). Furthermore, to evaluate the effect of soil characteristics on ant nest-site preference we analyzed pH, % organic matter and texture from: i) soils excavated by newly-mated queens; ii) nesting sites of mature colonies in three different areas of the Estate.
Preliminary results suggest a slight effect of treatments on queen survival and the existence of differences in the nesting-sites microhabitat characteristics possible involved in species coexistence.

Hybridogenesis through thelytokous parthenogenesis in two *Cataglyphis* desert ants

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Increased genetic variability among offspring is a major advantage of sexual reproduction. Some species have however evolved alternative unorthodox reproductive systems to benefit from both sexual and asexual reproductions, like hybridogenesis. Under hybridogenesis, both the maternal and paternal genomes are expressed in somatic tissues, while the germ line is purely maternal. Recently, a unique case of true hybridogenesis at a social level was reported in the desert ant *Cataglyphis hispanica* in which all workers are sexually produced hybridogens while new queens and males are produced through parthenogenesis. Thus, only maternal genes are perpetuated across generations (Leniaud et al., 2012). Here, we show that such an extraordinary reproductive strategy evolved in two multiple queens species of *Cataglyphis* belonging to the same phylogenetic group, *C. velox* and *C. mauritanica.* In both species, queens mate exclusively with male(s) originating from a different genetic lineage than their own to produce hybrid workers, while they use parthenogenesis to produce the male and female reproductive castes. As a consequence, all queens within colonies share the same multilocus genotype and never transmit their mates' alleles to the reproductive castes.

Leniaud L. et al. 2012. *Current Biology in press.*

Life-history traits as causes or consequences of polygyny

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Secondary polygyny, i.e. having multiple reproducing queens within the colony, has attracted much attention to understand how lowered relatedness influences colony social behavior and the potential for kin selection. However, polygyny is also recognized as a syndrome where a set of life-history traits change compared to monogyny, e.g. short distance dispersal of sexuals, short queen lifespan, low queen fecundity, and low queen-worker size dimorphism (Bourke & Franks 1995). These traits have important consequences for the demographic and genetic structure of the population, but their relationship is poorly understood as so far there has been almost no connection made between social insect biology and life-history theory (Cole 2009). We aimed to answer questions like: (1) Which life-history traits are causes and which are consequences of polygyny? (2) How do these traits affect the genetic structure of the population through changed dispersal and colony demography? (3) What habitat characteristics select for polygyny? To this purpose we implemented a spatially explicit, individual-based simulation model in NetLogo where ant queens are represented as individuals whose dispersal, survival, age, and reproductive output are tracked. Colonies can have any number of queens and reproduce by independent foundation or by budding. Survival and reproduction of the queens are dependent on worker number and competition among colonies for resources. The dispersal ability of gynes depends on the colony's investment of resources. The habitat is defined by level of stability and spatial heterogeneity of resources. Finally, mutations of life-history traits are allowed so that they can evolve under different ecological conditions, thus integrating demographic and genetic factors. We expect that by applying spatial structure and realistic colony life-cycles in our model will lead to an improved understanding of ant life history and how it may have shaped evolution of the polygyny syndrome.

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No inbreeding depression but increased investment in sexual reproduction in highly inbred ant colonies

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Inbreeding often leads to the expression of deleterious recessive alleles and consequently to a reduction in fitness. In Hymenoptera, these deleterious alleles are purged in haploid males, moderating the negative consequences of inbreeding. However, in these haplo-diploid species inbreeding results in another fitness cost: the production of sterile diploid males. We investigated the effects of inbreeding on the individual and colony level in the highly inbred ant *Hypoponera opacior*, a species with two annual reproductive phases including outbreeding winged sexuals and nest-mating wingless sexuals. Regular sib-matings led to high levels of homozygosity and the production of diploid males, which occasionally sired triploid offspring. On the individual level, body size increased in workers with inbreeding. On the colony level, productivity was unaffected by the degree of homozygosity, but inbred colonies invested more resources into sexuals than into workers. Workers might be able to assess the colony inbreeding level and alter their feeding behavior accordingly by raising more male larvae to adulthood and feeding diploid larvae better so that more diploid individuals pass the threshold of developing into a queen. Both larger worker body size and a higher queen/worker ratio can be explained by these changed investment strategies. Instead of suffering from inbreeding, these ants have developed strategies to regulate its extent and effects.

The proximate effects of temperature on bumble bee colony longevity and the foundation of a second annual generation.

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How pollinating insects are responding and will respond to higher environmental temperatures is a key topic in the study of ecosystem services in relation to global climate change. Increasing reports of winter-active bumble bees in their temperate range represent a possible example of such a response.. By manipulating the temperatures of colonies in controlled laboratory conditions, we sought to determine the proximate effect of temperature on several life history characteristics of the bumble bee *Bombus terrestris*. Firstly, we hypothesised that a higher temperature would increase the longevity of colonies. In order to test this, colonies of *B. terrestris audax* were reared at either 20 or 25 degrees Celsius to assess the effect of temperature on queen longevity, colony productivity and worker longevity. It was discovered that whilst temperature did have an effect on colony productivity, there was little or no effect on queen or colony longevity. Secondly, as an alternative explanation for the existence of winter-active bumble bees, we hypothesised that new queens would be more likely to establish a colony in the winter as a plastic response to warmer conditions (as suggested by the presence of a second annual generation of *B. terrestris* colonies in the species' Mediterranean range). We reared mated queens under different temperature treatments to reveal any effect of temperature on the probability of oviposition without diapause. We use the results of these studies to discuss the likely roles of these two mechanisms (colony persistence and second-generation founding) for the occurrence of winter-active bumble bees.

Do *Formica fusca* queens compete through egg quality*?*

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Colonies of many ant species permanently contain several reproductive queens (polygyny). The competition among queens for limited colony resources may result in conflict over personal reproduction manifested as unequal offspring contribution. Queens of black ant *(Formica fusca)* do not seem to compete directly, as is apparent from the absence of queen-queen aggression and social hierarchies, instead the observed differences in their reproduction seem to be largely the result of differences in their fecundity. Moreover the intrinsic properties of eggs such as their size and capability of early hatching may be contributing to reproductive differences among queens. Although differences in egg viability among queens are often suspected they have rarely been directly measured. We compared the size, timing of hatching and overall hatching success of eggs laid by queens of high and low fecundity. The results show that queens seem to allocate maternal resources differentially with the egg size inversely proportional to queen egg-laying rate. Although the onset and the length of hatching is the same, the overall hatching rate decreases with increasing fecundity of the queen. We are yet to explore the consequences of differential maternal investment into eggs on queen fitness by investigating the survival rate and competitive abilities of larvae as a function of their size at hatching and possible relationship between egg size and, larvae sex determination and caste development, to conclude whether queens do indeed compete with each other by regulating quality of eggs they lay.

Reproductive behaviour of the honey bee population in Ile de France: influence of the colonies in the drone congregation set up.

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Honey bee (*Apis mellifera*, L.) is a haplo-diploid social insect, living in colonies with a unique queen. The queen fertilization occurs at a defined drone congregation area, in mid-air. The queen can mate with an average of 15 drones. The black honey bee preservation centre, located in a 5km radius in the Rambouillet forest (40km south of Paris) has 120 bee colonies, including 80 colonies in the experimental apiary where we are working. So far, there has been very little information concerning the origin of the drones in the congregation. We still do not know if they come from the nearest colonies, or from other apiaries, in order to minimize the consanguinity in the next generations. To answer this question, we have sampled drones in a congregation during the 2010 and 2011 seasons. We have analyzed a total of 231 drones, including 183 in June 2010, and 48 in July 2011. We have genetically characterized the colonies from the experimental apiary, and the 231 drones with molecular markers. We also have counted the drone brood of each colony of the apiary every 15 days during these 2 years. The haplotype proportions observed in the drone congregation are correlated with the haplotype diversity of the experimental apiary. Changes made within the colonies between the 2 seasons are indeed found in the congregation in 2011. With the drone brood analyses, we have found a real link between the quantity of drones produced by a defined colony and the proportion of drones from this colony found in the congregation. Our results would be in favor of a close origin of the drones. So it is really important to have a sufficient genetic diversity in an apiary to limit the risks of consanguinity in the next generations fro consanguinity purposes.

Session 3. Disease, immunity, symbionts and social parasites

Molecular evolution of immune genes in socially diverse bees

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The evolution of sociality in ants, bees, wasps and termites has been substantially influenced by the elevated pathogen pressure in densely inhabited colonies. Social insects have evolved many collective defenses, i.e. social immunity, to complement the physiological individual-level immune responses. These include the use of antimicrobial compounds in the nest building material and collective recognition and removal of infected individuals. The effect pathogens have had on the evolution of genes involved in physiological immune system genes in social insects can be studied by comparing evolutionary patterns in immune genes of closely related social and solitary organisms. We have done this by studying sequence evolution of a large number of immune genes in bees with varying levels of sociality (highly eusocial, primitively eusocial and non-eusocial) using codon-based likelihood models of nucleotide substitution. There is surprisingly little evidence for positive selection in the contrasts done to date. This may suggest that social immunity acts as a buffering mechanism and reduce selection pressure on immune genes.

Diverse pathogen adaptation to individual *versus* social immunity in ants

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The interaction between hosts and pathogens is characterised by an ongoing coevolutionary armsrace, involving evolution of increased pathogen virulence followed by counteradaptations of the host's immune defences. In social insects, these host defences do not only include the hygiene behaviour and physiological immune systems of the individual group members, but also their collectively performed, social disease defences ranging from sanitary behaviours, use of antimicrobials and organisational adaptations. To disentangle the selection pressures on pathogens stemming from these two levels of defences, we performed a selection experiment, using the Argentine Ant, *Linepithema humile*, and the general insect fungus *Metarhizium* as a study system. We used pools of natural isolates (obtained by B.M. Steinwender, University of Copenhagen) of this fungus, which is an entomopathogenic obligate killer that infects both solitary and social insect species in nature. We exposed individual ants to the fungus and either reared them in isolation or in social contact to healthy nestmates. We then determined pathogen adaptation to the selection pressure under individual host defences (isolation treatment) or individual plus social immunity (group treatment), respectively. We found that the two selection regimes both led to increased killing rates over time, but that the dynamics of pathogen adaptation and fungal strain composition varied between the isolated versus group treatment. Our data reveal that the additional group level defences in insect societies add new selection pressures for their coevolving pathogens, thus affecting disease dynamics.

Social immunisation in the invasive garden ant

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Living in groups increases the risk of disease transmission and has thus led to the evolution of sophisticated immune defences in insect societies that depend on the cooperation of all group members, i.e. their 'social immune system'. In ant colonies, healthy nestmates help a pathogen-exposed individual e.g. by removing infectious particles from its cuticle via allogrooming behaviour. This is beneficial to the exposed individual as it reduces its infection risk. The helpers may pay a cost as they may contract the disease themselves. However, this seems to occur rarely. Instead, social contact to a pathogen-exposed individual can also directly benefit the helpers by reducing their susceptibility to the pathogen upon future encounters. So far, this phenomenon of 'social immunisation' has been described for three social insect - pathogen interactions, yet its broader prevalence and underlying mechanisms are little understood.
We tested whether social immunisation in the invasive garden ant *Lasius neglectus* against the fungal pathogen *Metarhizium anisopliae* is based on an active upregulation of the immune system of nestmates or their passive protection via the transfer of immune effectors from the exposed individual (the latter being the likely mechanism after bacterial infections; Hamilton C. et al. 2011. Biol. Lett. 7: 89-92). Using behavioural observations, immunological assays and immune gene expression studies, we found no evidence for the involvement of passive immunisation. Instead we show that, due to social contact, nestmates contract non-lethal low-level infections from pathogen-exposed individuals. These low-level infections cause an active upregulation of a pathogen-specific set of immune genes and ultimately result in a protective immune stimulation. We further analysed whether the ants are able to clear the observed low-level infections after longer periods of time and therefore may lose their protective immune stimulation.

High socio-spatial compartmentalization supports the organizational immunity hypothesis in honeybees

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Complex insect societies formed by thousands of individuals face the problem of efficient spatial and social organization among colony members. While highly connected networks are predicted to increase efficiency and group coordination, they are believed to also increase the rate of pathogen transmission within the colony. Diseases pressure is a major challenge for insect societies survival and theory predicts that contacts between individuals should be limited by spatial and behavioural compartmentalisation, giving rise to highly structured networks. We tested this "organizational immunity" hypothesis by recording at high resolution honeybees individual spatial and social behaviour on the comb. The social network and the spatial organization clearly separate nest mates in space and time and are primarily organised by age. Older workers (foragers) are at the periphery of the social network, while the colony core is formed by young bees and the queen. Connectivity and spatial overlap are high between same-age workers but low between different-age workers, thus creating a compartmentalized structure which limits the likelihood of disease spread between the periphery (foragers) and the core (queen/nursers) of the colony network. These results support the organizational immunity hypothesis, strongly suggesting that, beyond the ergonomics requirements, diseases pressure shaped colony organization in honeybees.

Alteration of social behavioural networks in ant colonies following pathogen exposure

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Social interaction networks in insect societies are shaped by colony-intrinsic factors such as density, age and task performance of the interacting individuals. In addition, extrinsic factors like pathogen exposure are known to change the behaviour of exposed individuals and also their nestmates. Ants respond to direct exposure with pathogens by selfgrooming, and also show allogrooming towards nestmates that are exposed. This self- and allo-directed grooming behaviour reduces the risk of infection of the exposed ant, but bears the risk of cross-infection to the previously healthy allo-groomers.
We analysed how exposure of single *Lasius neglectus* ant workers with the general insect pathogenic fungus *Metarhizium anisopliae* affects the social interaction network in the colony. We found that self- and allogrooming frequencies were altered in both the pathogen-exposed ant and its nestmates. Epidemiological modelling reveals that these behavioural changes are adaptive, limiting disease transmission in the colony. Interestingly, it is the alteration of particularly the social grooming between individuals that has highest effect on disease dynamics in ant colonies.

Young ant queens are attracted to fungal entomopathogens when choosing a nest site

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For a young ant queen, founding a new nest alone is extremely risky. One of the threats is the exposure to pathogens in potential nest sites, particularly for young queens that nest in the soil with no help of grooming workers. For these independent founding queens, an efficient line of defense may be to detect and avoid nest sites that are contaminated by pathogens. Here, we tested with nest choice assays if young *Formica selysi* queens were able to detect fungi in potential nest sites, if they specifically avoided entomopathogenic ones, and if they were able to discriminate between a live and a heat-killed pathogen. Surprisingly, we found that young queens did not avoid the common soil entomopathogens *Beauveria bassiana* and *Metarhizium brunneum*, but were initially attracted by them. The queens were similarly attracted by one of the non-entomopathogenic fungi tested, *Fusarium graminearum*. For the other non-entomopathogen tested, *Petromyces alliaceus*, the ants first did not show a preference, and were repelled by it by the end of the experiment. Moreover, the ants were not attracted by heat-killed *B. bassiana*. Together, our results suggest that ants are able to detect live fungi, and that they are attracted by some of them whether or not they are pathogens. Relatively low rates of mortality observed in our experiments indicate that in the conditions tested the cost of nesting in a contaminated site is quite low, at least with regard to the survival of the queens.

Do Ants Commit the Concorde Fallacy?

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Ants can detect brood infections and respond to them by grooming or removing infected individuals from the colony [1]. However, as yet no study has focussed on the lengths ants will go to prevent brood infections, and whether this differs depending on brood value. The Concorde Fallacy states that one should not invest further into a project because of large past investments, but rather the potential future gain. It can be applied to biological systems displaying behaviours that are not optimal [2]. To test whether ants commit the Concorde Fallacy, workers were presented with different stages of brood, inoculated with either *Metarhizium* sp. spores or a control. Each nest was then observed for a period of five days, and using scan sampling, the behaviour of each ant was recorded. This enabled us to determine the frequency of worker-larvae interactions directed at each different brood stage. Here we report how the frequency of antiseptic worker behaviour differs according to brood age and parasite treatment, and thus whether ants commit the Concorde Fallacy when protecting their future nestmates against parasites. In addition, we report a novel behaviour which we interpret as a new mechanism of defence against parasites. We discuss our results in the context of social immunity.

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Who performs undertaking behaviour in the ant *Cataglyphis velox*?

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The removal of dead bodies (undertaking) is an important hygienic task in insect societies, as bacteria and fungi grow out of decomposing cadavers and may infect colony members. Preventing disease transmission is particularly crucial in the long-lived colonies of social insects, in which closely related individuals live together in high densities. Insect societies have therefore evolved collective disease defences based on the cooperation of colony members, whereas organisms of solitary species have to rely only on their individual behavioural and physiological defences. Division of labour is a key property of insect societies and a basis for their ecological success. We therefore studied if division of labour also exists for the hygienic task of undertaking behaviour. Workers performing this task might be at high risk of pathogen infection due to cadaver handling and leaving the confines of the nest. At the colony level, it may thus be advantageous if a subset of colony members would specialise on this risky task. Using the thermophilic ant *Cataglyphis velox* as our study system, we repeatedly exposed colonies with nestmate cadavers and indeed found that cadaver removal is limited to only very few colony members in the majority of the studied colonies. We analysed whether repeated cadaver exposure affects individual and colony task performance. We also test if cadaver-handling individuals belong to a specific genetic subset of the colony (e.g. one specific matriline or patriline), and invest differently into their physiological immune system (measured as activity of the enzyme phenoloxidase, which plays an important role in insect innate immune responses).

The impact and dynamics of a multi-host nematode parasite of bumblebee queens

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Despite an upsurge of research into the parasites of social insects, remarkably little is known of the ecological impact of parasites on their social insect hosts. Furthermore, the vast majority of studies have examined single species interactions, whereas at least 50% of parasites have multiple hosts. Bumblebees provide an excellent system in which to address these questions, as they live in multi-species assemblages and host numerous parasites where the basic natural history is well-known. *Sphaerularia bombi* is a nematode parasite of bumblebee queens that acts to castrate queens and behaviourally manipulate them for its own advantage. Using field and laboratory data, we determine the evolutionary theatre within which the ecological impact of this parasite has evolved. We present data on the prevalence, virulence and fitness of this parasite across 4 host species. Using laboratory experiments we determine the timespan of parasite reproduction and then examine how parasite virulence fits with both biological and theoretical expectations. Finally, we examine the potential ecological impact of this parasite on bumblebee assemblages.

How disease-ridden are our pollinators? Honeybee pathogens in bumblebees (*Bombus* sp.) across the UK

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It appears the main beneficiaries of globalisation, after humans, are invasive species and infectious agents such as parasites and pathogens (Vitousek et al. 1996). Pathogen pollution and spillover events from traded livestock are, at a global scale, the major source for emergent infectious diseases that impact native species (Daszak et al. 2000). Trade in honey bees has been the key driver of emerging diseases in managed honey bee populations (Genersch and Aubert 2010). However, the parasites and pathogens behind these diseases pose a further threat, as they are increasingly being recognised as multi-host, rather than *Apis*-specific parasites. In particular, a range of these parasites have recently been detected in bumblebees (*Bombus* sp.) (Evison et al. 2012; Genersch et al. 2006). Even though potentially detrimental to the native bee populations, next to nothing is known about the basic epidemiology of the newly detected parasites in these vital and charismatic pollinators. We conducted a structured geographic survey across 26 sites in the UK, focusing on the prevalence of the microsporidian *Nosema ceranae* and the Iflavirus DWV (deformed wing virus) in bumblebees and honey bees to determine the dynamics of the parasite across the two host groups. We find the pathogens in bumblebees as well as honeybees across the UK, with strong spatial variation in prevalence. This suggests that studies are urgently needed to understand the infectivity and virulence of these pathogens in bumblebees to comprehend the threat they are facing.

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Ant workers die young and colonies collapse when fed a high-protein diet

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A key determinant of the relationship between diet and longevity is the balance of protein to non-protein (fat and/or carbohydrate) energy in the diet. Eating excess protein relative to non-protein energy shortens lifespan in solitary insects. Here we investigated the link between high-protein diet and longevity, both at the level of individual ants and colonies in black garden ants *Lasius niger*. We explored how lifespan was affected by the dietary protein to carbohydrate ratio and the duration of exposure to a high-protein diet. We show that: i) restriction to high-protein, low-carbohydrate diets decreased worker lifespan by up to ten fold; (ii) reduction in lifespan on such diets was mainly due to elevated intake of protein rather than the lack of carbohydrates; (iii) only one day of exposure to a high-protein diet had dire consequences for workers and the colony, reducing population size by >20%.

The effect of overwintering temperature on the diapause survival, remaining energy resources, and immune functions of *Bombus lucorum* queens

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Bumblebees are widely studied as important pollinators of both commercial crops and natural flowering plants. The increasing evidence of climate change suggests that winter temperatures in northern parts of Fennoscandia will rise anywhere from 4-7 degrees to the year 2050, getting more similar to southern European conditions. For cold adapted species such as the white-tailed bumblebee (*Bombus lucorum)* this can pose a challenge, since it may give an advantage for closely related southern species such as the buff-tailed bumblebee (*Bombus terrestris*) during diapause in survival and the amount of remaining spring resources (Sorvari J. et al. 2011. J. Insect Conserv. 15: 727-731). We experimentally studied the effects of overwintering temperature (0 - +3℃ = cold and +7 - +10℃ = warm) on the diapause survival, remaining energy resources, and immune functions after a 4 month period in *B. lucorum* (Moret Y., Schmid-Hempel P 2009. Oikos 118: 371-378). 150 native queens were collected from South Western Finland in early spring 2011. After rearing, 63 queens mated and started the overwintering experiment, group A (*n* =31) in cold (+0 - +3 ℃) and group B (n=32) in warm (+7 - +10℃) diapause conditions. We used fat body weight as a parameter of remaining energy resources**,** and phenoloxidase activity as a parameter of the immune functions of overwintered queens (Bradford M. M. 1976. Anal Biochem 72: 248-254). We found the threshold weight of survival for *B.lucorum* queens to be 0.4g, which is 0,2g lower than previously shown in *B.* terrestris (Beekman M. et al. 1998. Entomol. Exp. Appl. 89: 207-214). PO-activity and protein concentrations were found to be lower in queens at warmer diapause conditions, and they also had significantly less fat than in the colder diapause group queens. Larger individuals used more fat in cold, but not in warm diapause conditions. An increase in diapause temperatures in northern areas may lower the spring condition of local cold-adapted bumblebee species and especially lighter individuals seem to lose the advantage of small body size compared to cold conditions.

Disruption of trophobiosis between argentine ants and citrus mealybugs: anti-ant strategies adopted by a predator ladybird to eat undisturbed.

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Trophobiosis is a mutualistic association (common between ants and sap-sucking hemipterans) where one of the partners gives food in exchange for protection. Sap-sacking insects offer honeydew and the ants care for them in various ways, in particular giving protection against enemies. Our study model includes the argentine ant (*Linepithema humile*), the citrus mealybug (*Planococcus citri*) and a mealybug predator, the ladybird *Cryptolaemus montrouzieri*. One of the features making argentine ant a pest is its tendency to establish intensive relationships with honeydew-producing hemipterans defending them from predators. However, not always this paradigm of trophobiosis is respected. In fact, in previous field studies we collected evidences that the presence of ants does not affect the predatory effectiveness of the beetle on tended mealybugs. Here we report the results of a laboratory investigation aimed to clarify if the kind and abundance of food available for the ants can influence their reaction towards the ladybirds and which are the eventual morpho-functional and behavioural strategies allowing this beetle to be a specialized predator also on ant-tended coccids. Experiments were conducted on colonies fed with different diets (artificial diet, honeydew obtained from tended mealybugs, both diets). In order to quantify the reactions of the ants towards different stimuli we analysed their behavioural responses to: adults and larvae (with or without the typical waxy cover) of *C. montrouzieri*; potential prey; potential prey covered with the ladybird wax. Results showed that the diet does not influence the aggressive behaviour of the ants against the ladybirds. Moreover, the ladybirds seem to rely on several (behavioural, morphological, chemical) adaptations to limit or prevent the attacks by the ants. In particular, the waxy filaments that cover the body of larvae are very effective in this context. Possible mechanisms underlying these adaptations are discussed taking into account evolutionary, ecological and applicative implications.

Genomic analyses suggest metabolic complementary between fungus-growing termites, their mutualistic fungus and termite gut communities

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Fungus-growing termites (Macrotermitinae, Blattodea) are major decomposers in the Old World tropics, where they form some of the most complex colony and mound structures of any insect group. The Macrotermitinae live in mutualistic symbiosis with *Termitomyces* fungi (Tricholomataceae, Basidiomycotina), which aid in the degradation of plant material and are housed on a special substrate in the nest. This fungus comb is maintained by the termites through the continuous addition of partially digested plant material that has passed through the termite gut along with asexual *Termitomyces* spores. To explore symbiont complementarity, we sequenced the genome of *Macrotermes natalensis* and its *Termitomyces* symbiont, as well as its gut microbiomes (from a queen, soldiers and workers). As expected in an ancient obligate host-symbiont association, our findings support metabolic specialisation. Our metagenome analyses revealed that worker and soldier guts are rich in microbial OTUs and have the metabolic potential to complement *Termitomyces* and termite enzymes in the decomposition of plant biomass. In contrast, the queen gut harbours a markedly reduced bacterial community, and the almost complete absence of plant decomposition enzymes suggesting that the queen's diet consists of *Termitomyces* material only. Our findings confirm functional complementary in the *Macrotermes*-*Termitomyces*-gut microbiomes, and provide evidence for caste-specific gut microbiomes with queens receiving higher quality food that workers and soldiers.

Resistance to and tolerance of parasitic large blue butterflies by *Myrmica* ant colonies

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Large blue butterflies (*Maculinea* sp.) are virulent social parasites of *Myrmica* ant colonies that either directly feed on the ant brood or manipulate worker ants to feed them at the expense of the brood. The pattern of infection in the field suggests that *Myrmica* ant colonies differ in how susceptible they are to parasitism by large blue butterflies (i.e. they vary in resistance), but it also appears that there may be differences in the impact that the large blue caterpillars have on the fitness of their host colony (i.e. the colonies vary in their tolerance of the parasites). Here I review the evidence for resistance and tolerance, and examine some of the factors responsible for both, concentration on the association between *Maculinea alcon* and its hosts in Denmark. Resistance to infection appears to depend primarily on the ability of the parasites to match the cuticular hydrocarbons of the host colony (Als *et al.* 2001, Nash *et al.* 2008), and on the social structure of the host (Nash & Boomsma 2008, Fürst *et al.* 2012). Resistance once infected varies between host species (Nash *et al.* 2011), and may also depend on social structure (Nash & Boomsma 2008). I will present new data on differences in tolerance between host species.

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Nash *et al.* 2011. Ins. Soc. 58: 391-401

Comparison of chemical deception strategies and acoustical mimetism between two social parasites of ants

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Butterflies of the genus *Maculinea* are among the best documented examples of myrmecophilous insects. They are obligate parasites, strictly depending on the ants classified in the *Myrmica* genus. All *Maculinea* species have a phytophagous early larval phase, but once the fourth larval instar is reached, larvae drop to the ground where they are “adopted” by *Myrmica* ants. *Maculinea* butterfly complete their development during the last larval instar in an ant nest. The level of the parasites' integration within the host colony results from two distinct strategies. In the so-called "cuckoo" species, *Maculinea* larvae become perfectly integrated members of the colony and are actively fed by worker ants by food regurgitation (trophallaxis). In contrast, larvae of other species, known as "predator" species, actively feed on the ants' brood. In all cases, however, the larvae are attended by ants until pupation. To achieve social integration within the ant nests, the caterpillars mimic the acoustic signals as well as the cuticular hydrocarbon profile of their host ants.
In our research, we focused on two sympatric butterfly species, *Maculinea alcon* (cuckoo species) and *Maculinea teleius* (predatory species) which exploit the same host ant species *Myrmica scabrinodis*. We compared cuticular hydrocarbons of host ant with chemical profiles of the pre-adoption and post-adoption *Maculinea* larvae to explore the chemical patterns at the base of parasite integration and to verify the existence of host manipulation. Moreover, we investigated the meaning of acoustics signals and we try to understand if a trade off between chemical and acoustical signals exists and if it depends on the level of integration inside host colony.

Temporal and spatial patterns of ant social parasite segregation

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Myrmica ant colonies host numerous insect species, including the larvae of *Maculinea* butterflies and *Microdon myrmicae* hoverflies. Most previous studies on the interactions between *Maculinea* species or *M. myrmicae* and their *Myrmica* host ants have focused on ant host specificity patterns, or on the chemical and acoustical mimicry implemented by *Maculinea* caterpillars. Most of these investigations have dealt with the interactions between a single social parasite and its host ant. Studies on social parasite communities are scarce, particularly as concerns those addressing potential competition among parasites.We investigated sympatric populations of social parasites to assess the strategies allowing them to share the same pool of resources (i.e. *Myrmica* colonies). We carried out our study at five wet meadows with *Molinia* spp., each of which was habitat for various combinations of four social parasites (i.e. *Maculinea teleius*, *M. nausithous*, *M. alcon* and *Microdon. myrmicae* hoverflies). Of these parasites, we investigated the spatial distributions, host segregation and temporal overlapping in colony resource exploitation. We found that in some places social parasites were segregated in space, whereas in some other cases they differed in host species. In three cases, we found *Myrmica* colonies infested by more than one parasite species and hosting a high number of parasite larvae. These data suggest the some ant colonies are more prone to be infested by social parasites. The most common parasite association observed was between *Maculinea teleius* and *Microdon myrmicae.*

The social parasitic wasp *Polistes sulcifer* is not able to suppress host workers ovarian development: what does it tell us about workers reproductive decision?

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Social parasites face the challenge of getting accepted into the host colony and successfully exploiting its brood care. More specifically, parasites would benefit from functionally replacing the former breeder of the colony in order to inhibit worker reproduction and redirect their parental care toward its brood. On the other hand, host workers would benefit from detecting the parasite presence and investing into either direct or indirect fitness, i.e. laying eggs and/or rearing related workers' brood. Investigations on different model systems give contrasting evidences. In some cases the parasite prevails, in others the host workers do. We performed a laboratory study on a host-parasite social paper wasp system, *Polistes sulcifer* (parasite) - *Polistes dominulus* (host), to determine whether the parasitic queen suppresses host workers reproduction. Previous studies suggest that the parasite queen has complete control over host workers reproduction. However, by comparing worker reproductive efforts in parasitized and unparasitized colonies we show that the former invest more in direct reproduction than the latter. They have more developed ovaries and lay more eggs when opportunities arise. This worker investment in reproduction in parasitized colonies is manifest 40 days after usurpation but some evidences suggest it occurs soon after the parasite takes over the colony. This result suggests that in this species, contrary to previous beliefs, the parasite is not able to completely replace the host queen. It also indicates that our comprehension of the factors regulating worker reproductive decisions in these wasps is still incomplete.

The role of workers and host choice in a fungus growing ant social parasite

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The internal environment of social insect colonies is attractive to parasites, as the host workers provide protection against predators and diseases, and the colonies contain valuable resources. Inquiline social parasites are dependent on being fully integrated into the host colony for much of their lives in order to reproduce. Most inquiline ants have completely lost their sterile worker caste. An exception to this is *Acromyrmex insinuator*, a social parasite of the fungus-growing ant *Acromyrmex echinatior*. In this study we investigate the ratio, distribution and location of *Acromyrmex insinuator* workers by sampling workers from four different parts of the host colony (the top, middle and bottom of the fungus garden and the foraging area). We hypothesized that there will be more parasite workers in the middle part of the fungus garden which contains most nutrients, and where the social parasites may be best able to alter the development of host workers and reproductives. Furthermore we demonstrate that parasite queens can distinguish and prefer already parasitized host fungus. By infiltrating already parasitized colonies the queens will be accepted more easily and can increase their reproductive fitness, as studies have shown that multiple parasite queens in the colony enhance the suppression of host reproduction and thereby promote faster production of parasite sexual.

Effects of socially parasitic *Myrmica rubra* microgynes on host colonies under laboratory conditions

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Microgynes of the ant *Myrmica rubra* (*M. microrubra* Seifert 1993) are thought to be incipient social parasites and potential examples of sympatric speciation (Savolainen & Vepsäläinen 2003). Although not a separated species from their host (Steiner et al. 2006, Leppänen et al. 2011), microgynes are usually genetically differentiated from their local host populations (Vepsäläinen et al. 2009). Microgynes do not produce many workers and do not occur independently from their macrogyne hosts (Seifert 2010). This suggests that they are recently evolved obligatorily parasitic inquiline lineages rather than alternative reproductive morphs. Inquilines harm their hosts by causing investment into non-related instead of own offspring as well as by brood predation (Passera et al. 2001). This study aims to directly measure virulence and infectivity of microgynes in a controlled laboratory experiment. Naturally parasitized experimental colonies, non-parasitized control colonies and pure microgyne colonies were kept under identical conditions. Other experimental nests were artificially infected with microgynes from foreign colonies by dropping individuals into the host foraging arena. Macrogyne host queens in naturally parasitized colonies died significantly earlier than queens in non-parasitized and experimentally infected nests. More than half of the microgynes survived during the first five months when artificially introduced into foreign colonies. In infected nests, the presence of microgynes decreased the number of worker pupae produced compared to control nests. These results indicate that the microgyne form of *M. rubra* is a social parasite with moderately negative effects on colony growth but high potential for transmission by quick integration into new host colonies. It further seems that they preferentially exploit older host colonies in their natural environment.

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Should I stay or should I go? Drifting behaviour as a reproductive decision in workers of the bumble bee *Bombus terrestris*

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During the last decade, colonial drifting from workers has repeatedly been observed in bees, wasps and bumblebees and considered a form of intraspecific social parasitism. The evolutionary relevance of these observations is however not yet fully established since they result, for the most part, from experimental situations that could have artificially promoted this behaviour. In the bumblebee *Bombus terrestris* the factors involved in worker reproduction in their nest are now well known. Conversely, those that could trigger the drifting behaviour are completely unknown. The aim of this study is to characterize at the individual level the proximal factors which may promote drifting behaviour to determine if this behaviour has ultimately been shaped to function as an alternative reproductive strategy. We experimentally manipulated the fertility of workers in colonies at different developmental stages and then installed these colonies in an open environment and finely quantified the drifting behaviour of workers and their reproduction in the different nests. The analysis of movements between nests shows that, regardless of the colony stage, workers which leave their nest drift more often when they are fertile. Moreover, these individuals stay longer in heterocolonial colonies and often lay eggs. Our results also show that the influence of worker fertility on drifting behaviour is modulated by colonial context. The fertile workers move from their nest significantly more when their colony is in the competition than in the social phase. This study demonstrates for the first time that individual factors related to reproduction promote drifting behaviour. The importance of these factors in the drifting behaviour is consistent with the hypothesis that this behaviour is a true reproductive decision by workers of *Bombus terrestris*. The differences observed in the initiation of this behaviour according to the colonial phase are discussed in terms of cost-benefits associated with each reproductive decision.

Antiseptic homes: from solitary to advanced eusocial wasps the nest turns into a social immunity tool

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Group living leads to an increased risk of disease transmission. As a result of coevolutionary dynamics between parasites and hosts, social insects have evolved the ability to counteract microorganisms epidemics primarily by the use of antimicrobial secretions. Further, beyond the individual immunity, social insects colonies benefit from the fact to be many to mount collective defences (phenomena that has been summarise as parts of a "social immunity"). In bees, wasps and thrips, the evolution of sociality was accompanied by the evolution of stronger antimicrobial compounds which progressively increased in efficacy with the enlargement of group size. Here we hypothesize that as colony size and sociality level increased, chemical defences against diseases turned from simple mechanisms of individual immunity to complex forms of social immunity. Using Vespidae family as model, we found that nest, a simple workplace for adults and a nursery for brood in solitary and facultatively social species (Eumeninae and Stenogastrinae) turns into an antiseptic tool in the primitive and advanced eusocial species (Polistinae and Vespinae). Our findings suggest that in the evolutionary transition toward more complex societies the natural selection turns from individual selection to colony level and multilevel selection.

How internal state and social cues affect colony preference in the context of social parasitism in the bumblebee *Bombus terrestris*

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In the primitively eusocial bee *Bombus terrestris* some workers drift from their colony to a foreign conspecific colony where they can lay haploid male eggs. However little is known about the selective pressures which have shaped this form of intraspecific social parasitism. A detailed understanding of the factors involved in this decision-making is therefore necessary to be able to bridge this gap. For example, what are the cues workers use in their reproductive decisions to find and enter an unrelated nest? Recent studies show that a combination of individual and social factors is involved in the decision to leave the nest and hence a similar combination of factors probably influences the choice to join a conspecific colony. To test this hypothesis, we set-up a controlled laboratory experiment to study the effect of internal state (variations in workers' ovarian development) in a choice test between two kinds of wax, one coming from their own colony and the other from an heterocolonial one. Wax contains colony-specific cues and it may play a role in colony recognition and when a worker chooses a foreign colony to drift in. We also tested the possible influence of the social environment on wax preference by testing workers originating from colonies in social and competition phases. The worker's interest for the two stimuli was finely quantified using a tracking software and a posteriori dissections were done to measure the ovarian development of all tested individuals. This study provides data about wax discrimination in colonies of bumblebee, and sheds light on some proximate aspects of worker social parasitism.

Lack of evidence for local adaptation suggests uniform coevolution in a paper wasp social parasite-host system

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The continuous process of reciprocal adaptations and counter-adaptations between parasites and their hosts often lead to coevolutionary dynamics. The geographical mosaic theory of coevolution (Thompson, 2005) predicts that in spatially structured environments the parasite-host arms race could follow different trajectories leading to spatial localized patterns of coevolution. A geographical mosaic of coevolved phenotypes could thus occur where the parasites/hosts are locally adapted to their sympatric counterparts, showing higher fitness on sympatric than on allopatric hosts/parasites. In contrast with this geographically localized scenario of coevolution, parasite-host arms race could follow the same trajectory across all locations, and no evidence of different performance of parasite/host across the geographic scale should be found (Foitzik et al., 2001). We addressed the "localized vs uniform coevolution" issue in a social parasite-host system in paper wasps. We performed laboratory cross infection experiments to compare parasite's performance between sympatric and allopatric combinations of parasite-host populations. We estimated several parameters of parasite's fitness measuring, from the infection outcome to the final reproductive success of parasites. Our results demonstrated that parasite's performances do not change according to whether the opponents occur in sympatry or allopatry, which shows that the parasite species, *Polistes sulcifer,* is not locally adapted nor maladapted to its host, *P. dominulus*. This result suggests that in this parasite host system the coevolutionary arms race is spatially uniform.

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Ant community structure of syntopic populations of *Maculinea alcon* and *Maculinea 'rebeli'* (Lepidoptera: Lycaenidae)

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The lycaenid butterfly *Maculinea alcon* and its taxonomically disputed form *M. 'rebeli'* are obligate parasites of *Myrmica* ant species. Both forms apply „cuckoo" parasitic strategy: they are directly fed by ant workers. Yet they have different host plants, and different flight periods. There is a single known location in Europe, where they occur syntopically, which is at Luna de Jos (Cluj County, Romania) in a semi-dry meadow. Both form's host plants (*Gentiana pneumonanthe* and *G. cruciata*, respectively) occur there *Gentiana pneumonanthe* (main host plant for *M. alcon*) and *G. cruciata* (main host plant for *M. 'rebeli'*) present. The flight period of both butterflies is clearly separated, while their most common host ant species is *Myrmica scabrinodis*, although other *Myrmica* species could also serve as hosts. We investigated the ant community of the meadow, where both forms occur syntopically, and the relationship between the ant community structure and the distribution of butterfly eggs. The study area was sub-divided in wet and dry patches, which also correlated with the presence of *G. pneumonanthe* and *G. cruciata*. *Formica pratensis* was the ant species present in the highest abundance. Four possible host ant species occurred in the area, of which *Myrmica scabrinodis* was the most abundant. We found no significant differences between the two patch types regarding the ant community structure. There was also no significant relationship between the abundance of host ant species and the abundance of butterfly eggs.

Factors conditioning the success of the different individual reproductive strategies available to bumble bee workers

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An interesting feature of insect societies resides in the great diversity of individual reproductive strategies. In the annual bumble bee *Bombus terrestris,* in addition of the well-known intra-colonial competition where workers can reproduce inside their nest at the end of the colonial cycle, workers also have the possibility to leave their nest and to reproduce in another conspecific nest. Workers thus have two reproductive options and the consequences of each of them on the potential reproductive success at the individual level are unknown. Indeed, reproductive constraints differ depending on the developmental stage of the colony and these may also vary for a worker in an unrelated colony. The aim of this study is therefore to finely investigate in semi-natural conditions the impact of the social environment on the fertility, the possibility of reproducing and the reproductive success of workers who stayed or left their natal nest.
To do this, we experimentally manipulated colonies at the two different stages of the colonial cycle (social and competition phases) by introducing homocolonial workers with known fertility and then placed these colonies in an open environment allowing workers to move freely between nests. In one of the 3 sessions, egg-laying was monitored and all the emerging males were genotyped. In the two others, all the manipulated workers were collected after 7 days then dissected to check their ovarian development.
The results show that workers' ability to maintain their reproductive potential and to reproduce is dependent on their decision to stay or to move to another nest as well as on the development state of the colony in which they attempt to reproduce. The importance of the reproductive success of workers who opted for the different strategies is then compared and discussed.

Association of *Aphaenogaster subterranea* (Hymenoptera: Formicidae) with the nymphs of *Reptalus panzeri* (Hemiptera: Cixiidae)

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The association between ants and honeydew-producing hemipterans is well known, and there is an extensive literature on such relationships, involving mostly aphids, scales, mealybugs and treehoppers (Delabie, 2001). Those with cixiid planthoppers, however, are rare and based mainly on mere observational data, indicating only the existence of (possible) associations (e. g. Myers, 1929; Thompson et al., 1979; Neece, 1980).
Here, I report on a novel ant-cixiid relationship between *Aphaenogaster subterranea* (Latreille, 1798) and the nymphs of *Reptalus panzeri* (Löw, 1883). The aim of the study was to estimate the frequency of occurrence of planthopper nymphs in ant nests, and to make preliminary observations on the nature of this relationship.
Field work was carried out in a sub-Mediterranean oak forest in mid-western Hungary. Ant nests were mapped and excavated in 5 x 5 plots, each 3 x 3 m^2, in five sampling periods between May and September 2011. During nest mapping, various parameters were recorded, including the estimated size of ant colonies and the presence, number and estimated size of planthopper nymphs inside the ant nests. Laboratory work was also carried out to examine basic behavioural interactions between ants and planthopper nymphs.
With the exception of a few cases, the nymphs of *R. panzeri* were recorded only in the nests of *A. subterranea*, or were accompanied by the foragers of this species from neighbouring colonies, suggesting an obligatory association with the ant partner in this habitat. The relationship between the two species seems to be based, however, only on cohabitation, without further evidence of trophobiotic interactions, although the absence of escape behaviour of nymphs and that of the predatory or agonistic behaviour of ants indicate some kind of mutualistic relationship between them.

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Selective response of *Formica cinerea* Mayr (Hymenoptera: Formicidae) to corpses of different origin

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The social life involves major health risks, because the intensive contacts among individuals contribute to the fast spread of parasites and diseases. Among the defensive strategies, the most effective one is the collective disposal of corpses. These corpses might be used during interspecific conflicts, as the appearance of masses of dead corpses, could have negative influence on the behaviour of the attacked ant nest (Czechowski et al. 2009). That also implies that ants could recognize corpses of different species and react differentially. We examined in natural conditions the reactions of *Formica cinerea* to the presence of corpses of co-occurring rival species. The field-study was carried out in an open sandy pine forest area of the Kampinos National Park, Poland in August 2011, where altogether 10 *F. cinerea* nests were studied. Different combinations of freeze killed corpses were presented to the focal nests on plates at 0.5 m from the nest area border: corpses of nestmates, non-nestmate conspecifics, *F. fusca*, *F. polyctena*, *F. sanguinea* and its *F. cinerea* slaves. We recorded the ants' reaction to corpses and the time elapsed to the appearance of corpse carrying behaviour. The majority of corpses were carried inside the nests. *F. cinerea* clearly responded selectively to corpses of different origin: corpses of slave *F. cinerea*, the slave-maker *F. sanguinea* and the territorial *F. polyctena* were carried significantly sooner into the nest than corpses of the submissive *F. fusca* or nestmate and non-nestmate conspecifics. The nature and intensity of *F. cinerea*'s reactions seem to be in concordance with the nature of its interactions with these species. We presume that the corpses carried inside the nests were mostly consumed. The study was carried out within a joint research programme of the Polish and Romanian Academy. The financial support by the TÁMOP-4.2.2/B-10/1-2010-0012 fund is gratefully appreciated**.**

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Examining the "evolution of increased competitive ability" hypothesis in response to co-evolved and general parasites in the invasive paper wasps *Polistes dominulus*

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Successful invaders often manage to get established in a new range because they outcompete native sympatric species whose niche they eventually gain. This is generally achieved thanks to a higher fitness, which can be obtained by shifting resources to growth, fecundity and survivorship from other physiological compartments [1]. Since invasive species are usually released from their traditional enemies [2] (and they are initially not targeted by new ones) it has been hypothesized that immunity is a compartment which can be reduced for this purpose [3]. In this study, we aimed to uncover whether *Polistes dominulus* paper wasps, which invaded North America from Europe about 30 years ago, have developed an increased competitive ability at the expenses of a reduced immunocompetence. To explore this scenario, we compared wasp populations from the native and invasive ranges and we tested their susceptibility towards co-evolved and general parasites, at both the individual and colony levels. First, we examined the response to the specific coevolved parasite *Xenos vesparum* (lost after invasion) in terms of individual host susceptibility and hygienic behavior of nestmates. Second, we explored the response against general parasites by quantifying the bacterial clearance in individual wasps after a challenge with *Escherichia coli* and the hygienic behavior of colony members after a challenge with the fungus *Beauveria bassiana*. Our results show that American invasive *P. dominulus* have evolved higher response against *X. vesparum* at the colony level, but at the individual level their susceptibility is not significantly different from native conspecifics. Moreover, invasive *P. dominulus* display lower defense against general parasites at both the individual and colony levels. These findings suggest that in invasive species there is indeed a shift in the resource allocation for immunocompetence, but the responses against coevolved and general parasites might follow different/opposite evolutionary pathways which are not always easily predictable.

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Evolution of immune genes in the invasive Argentine ant

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The introduced populations of Argentine ant (*Linepithema humile*) are one of the most extreme examples of unicoloniality. In contrast, Argentine ants in South America, their native habitat, form smaller colonies which are genetically diverse and differentiated from each other. Introduced populations are genetically depauperate and homogeneous and form supercolonies that can extend for thousands of kilometers. High population densities, new pathogen fauna and reduced genetic diversity create potential selection pressures on the immune genes in the invasive populations. We aim to investigate how invasions have affected the evolution of immune genes in the Argentine ant. The genetic variation of selected immune genes will be compared between the native and introduced populations of *L. humile* and a sister species *L. oblongum*. We predict that if changes in population structure are the main drivers of molecular evolution both immune and control genes evolve faster in introduced than native populations. Alternatively, if changes in pathogen fauna are the main driving force of molecular evolution we expect that immune genes evolve faster than both the matched control genes in invasive populations and the immune genes in native populations. Fifty known candidate immune genes covering cellular response and all the four signaling pathways of the humoral response (Toll, Imd, JNK and JAK/Stat pathways) will be selected from the manually annotated immune genes of *L. humile*. In addition, 50 non-immunity genes physically close to each of the 50 immune genes will be included. The selected gene loci will be amplified by PCR from each individual and sequenced using Illumina sequencing. The sequence data will be used to assess recent selection in the immune genes of *L. humile*. The results will provide information on changes in immune genes associated with invasions and reveal the potential short-term adaptive evolution of immune genes in ants.

Facultative endosymbionts influence on sugar and amino acid composition of honeydew *Aphis fabae*

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Symbiotic interactions between animals and microorganisms are ubiquitous in nature. Nevertheless, the effects of endosymbionts on their host's metabolism are often poorly documented. Two secondary endosymbionts (genera Hamiltonella and Regiella) were found to occur facultatively in aphids. In order to determine the impact of the endosymbionts on the aphid's metabolism and the composition of their honeydew, we established two genetic lines of *Aphis fabae* infected with one of several strains of these endosymbionts. The honeydew sugar and amino acid composition was subsequently analysed using HPLC. Results are not yet available but will be presented during the congress. In recent studies both endosymbionts have been linked to an increased resistance to parasitoids, although their low prevalence in nature would suggest a cost of harbouring these endosymbionts (Vorburger *et al.* 2009). Honeydew is important for ant-tended aphids, so any considerable change in honeydew composition could interact with other symbiotic and coevolutionary interactions.

Vorburger et al. 2009. Evolution. 63(6): 1439-1450.

Analysis of an Exon Cassette of a Hyperdivers Immune Gene in Ants

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In insects, the Down syndrome cell adhesion molecule (Dscam) shows extraordinary genetic diversity through alternative splicing. More than 12000 alternative splice variants can potentially be transcribed in the honey bee *Apis mellifera*. Its function has been shown to lie in processes as different as brain development and immunity through facilitating cell-cell interactions. To understand better the function and evolution of Dscam, this gene is studied in ants from four different subfamilies. The sequences for 14 ant species were obtained through databank searches or sequencing. We used a Bayesian tree-building algorithm to construct a phylogenetic tree, which we based the selection model testing on. Using a comparative codon-based model, the results indicate the adaptive evolution of the exon cassette 4 in the analyzed data set through a better fit of a model with a selection component than one without. Comparing Dscam from ants with other insects corroborates previous studies demonstrating high similarity of this gene across insect orders and suggests a conserved function of this exon cassette. While most of the codons are under strong purifying selection, we find some evidence for positive selection (selection for change) in a few sites in Ig domains. Given the usual function of Ig domains in protein-protein interactions these few changes may be sufficient to alter the particular adhesive properties of the protein.

Microbial activity in wood ant nest and its role in ant thermoregulation and nutrient cycling

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Wood ant nest represent favorable condition for microbial activity in comparison with surrounding forest floor namely due to more suitable pH, higher temperature, and availability of nutrients and easily degradable organic material coming from food residues and ant excreta. This cause increasing of microbial numbers and biomass as well as alternation in microbial community composition.
Measurements of enclosures containg ant nest material with and without ants show that microbial respiration can contribute to 25% of ant nest respiration. In wet nest microbial activity can produce additional head that several fold increase heat energy production by ant itself. However this is enabled by high nest moisture which at the same time increase thermal capacity and heat conductivity so there is no overall effect in terms of nest temperature. Microbial activity can speed up litter decomposition in ant nest several time in comparison with surrounding forest soil and increase release of nutrients several fold. Ant effect on microbial activity wary with litter quality and nest moisture.

Session 4. Biodiversity, Community ecology, invasion biology and impact on human affairs

Comparative molecular and spatial analysis of rare and common bumblebees

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Bumblebees are among the most important insect pollinators of a number of arable, fruit and seed crops, however their populations are in sharp decline due to habitat loss and changes in farming practices, which has reduced the numbers of plants the insects forage on. We present data on the fine-scale spatial genetic structure of nest-founding queens and foraging workers, as a tool for modelling of landscape data, to predict the impact of habitat structure on queens and workers, forage and nesting resource distribution, and estimate bumblebee abundance. Here we focus on four common and one rare UK bumblebees that vary in aspects of their ecology. We sought to determine whether differences in genetic variation could account for the stark differences in abundance between species. Using microsatellite markers we reconstructed sibships for 2500 queens and workers collected in an agricultural landscape structured by sets of experimental agri-environment scheme options. We detected fewer workers per colony in the declining species than the abundant ones, and lower overall levels of allelic variation. This allows us to estimate ongoing loss of genetic diversity underlying global bumblebee decline. We discuss how the integration of the spatial genetic distribution of individuals from multiple pollinator species across the landscape can provide invaluable information on how habitat type influences pollinator abundance. We anticipate that this kind of data will be extremely valuable in informing the targeting of strategies such as agri-environment schemes for reversing bumblebee declines.

Double whammy? Impact of parasites and pesticides on bee fitness.

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Bees foraging in agricultural landscapes are likely to be exposed to a range of stressors, such as pesticide residues and parasites. There is evidence that both pesticides and parasites individually have an impact on the survival and fitness of bees, but in a natural setting, it is much more likely that bees will be exposed to several of these stressors simultaneously, with important implications for the bees' fitness. It is therefore vital to consider the potential interactions between stressors. Using *Bombus terrestris* as a model species, we have investigated the combined effects of exposure to a commonly used pesticide, and a widespread bumblebee parasite. The pyrethroid insecticide lambda-cyhalothrin was applied at field relevant doses to pollen, and fed to laboratory based *B. terrestris* colonies on a weekly basis. Adult workers, which had been exposed to the pesticide treatment throughout their development, were then exposed to the trypanosome parasite *Crithidia bombi*, and the development of infection, and longevity of the worker hosts monitored. Reproductive females (gynes) from these colonies were mated and exposed to *C. bombi*, and their survival through hibernation, and success at rearing a colony is currently being monitored. The results of these experiments have direct implications for wild foraging bee populations, many of which are in decline. I will discuss the progress of these experiments, and their relevance in a wider context of bee survival and fitness in natural environments.

Population genetics of source and reintroduced populations of the socially parasitic Large Blue butterfly *Maculinea arion*

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The Large Blue butterfly (*Maculinea arion*) is an obligate social parasite of *Myrmica* ants, and is specialized on *Myrmica sabuleti.* Populations of the butterfly are highly fragmented as it can only exist where the distributions of its host ants and larval food plants overlap. The species went extinct in the U.K. in 1979 and was subsequently reintroduced between 1983-1992 using individuals from the Swedish island Öland. The levels of genetic diversity and effective gene flow among populations are important to incorporate in conservation strategies as they affect the evolutionary potential of species. Therefore, we used fourteen microsatellite markers to 1) characterize the population genetic structure of 128 individuals from the poorly studied *M. arion* population on Öland, 2) analyse the levels of genetic diversity of 59 individuals from five of the introduced populations in the U.K.. We found that the level of genetic diversity in the reintroduced and the source populations were relative high and not significantly different. The reintroduced population had several private alleles and might therefore represent a unique subset of the genetic diversity in the North-Western populations of the species. We found significant genetic differentiation between the source and reintroduced populations, and among the reintroduced populations only a few kilometres apart. This is an important finding for the management of the species in the U.K. and has implications for future reintroductions of this threatened species.

How the West Was Won: molecular phylogeny and evolution of the holarctic termites genus *Reticulitermes*.

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Subterranean termites of the genus *Reticulitermes* are widespread across the entire holarctic region with more than 30 species and subspecies. While phylogenetic relationships have been deeply analyzed at a regional scale, with particular emphasis on the South-European range, a global picture is still lacking. Here we provide the first of attempt of a Holarctic-wide phylogenetic analysis of the genus, with the aim to reconstruct the phylogeny and the phylogeographic patterns leading to the present-day distribution. Three mitochondrial genes (COII, ND1 and 16S) have been analyzed with Maximum Likelihood and Bayesian Inference algorithms; moreover, cladogenetic timing has been estimated by calibrating Bayesian tree with fossil records and paleogeographic events. Analyses gave congruent results: the Japanese *Reticulitermes speratus* is placed basal to a dichotomy between two well-supported clusters embodying Balkanic and Middle-East taxa, one side, and Western-European and North-American species, the other side. Accordingly to the most common Holarctic's colonization routes, the most likely phylogeographic hypothesis is the westward spreading from the East Palaeoarctic region. After a single wave moved to Western Palaeoarctic, a lineage break occurred between a southern-heading clade (that will give rise to Balkanic and Middle-East taxa) and westernmost clade (made up by ancestors of North-American and Western-European species). Then a trans-continental migration occurred across the Thulean Bridge (connecting North America and Western Europe via Greenland-British islands), that lasted until 50 Myr ago, during the second Eocene thermal maximum. Accordingly to fossil estimates, the differentiation between North-American and Western-European *Reticulitermes* taxa was already present ~37.7 Myr ago. On the whole, data presented here depict a clear-cut evolutionary history of the genus *Reticulitermes* in line with main biogeographic patterns observed for holarctic fauna.

Genetic bottlenecks do not increase invasiveness in natural populations of the invasive garden ant *Lasius neglectus*

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Previous studies on the invasive Argentine ant *Linepithema humile* have shown that laboratory colonies become more aggressive towards conspecifics when genetic diversity, and consequently the diversity of chemical recognition cues such as cuticular hydrocarbons, is reduced (Tsutsui et al. 2003). Hence, in an encounter the least diverse colony is more likely to be the aggressor, thus conferring a competitive advantage. This raises the hypothesis that invasive ability mediated by such polarized aggression paradoxically may increase over time in introduced populations when colonies go through more genetic bottlenecks. This hypothesis was tested in natural populations of the invasive garden ant *Lasius neglectus* representing three independent introductions into Europe (Ugelvig et al. 2008). At each location we collected live ants from the oldest and largest population and from a young and small population to represent different invasion stages. Behavioural tests were performed to detect polarized aggression among *L. neglectus* populations (intraspecific), and to detect differences in aggression towards three other local ant species that are likely competitors (interspecific). Furthermore, the genetic (11 microsatellite loci) and chemical (cuticular hydrocarbons) diversity of populations was analyzed. Young and old populations did not differ significantly in genetic diversity or in aggression towards either conspecifics or local ant species. Furthermore, the genetic diversity did not correlate with aggression or the variation of cuticular hydrocarbons. This indicates that polarized aggression mediated by genetic bottlenecks is not an important factor increasing invasiveness in introduced populations of this species. However, populations differed significantly in other aspects of their aggression behaviour and in the average size of workers, suggesting variation in their ability to invade. This may have implications for how *L. neglectus* is being managed in areas where it is currently spreading.

Tsutsui N.D. et al. 2003. Proc. Natl. Acad. Sci. USA 100: 1078-1083.
Ugelvig L.V. et al. 2008. BMC Biology 6: 11.

Population genetic structure and range expansion of the tropical fire ant Solenopsis geminata in the Galápagos Archipelago

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Majority of the flora and fauna of the Galápagos Archipelago (Ecuador) are highly endemic, meaning that they evolved and live only in these islands. Therefore, introduction of exotic species in these fragile and sensitive environments leads to major ecosystem alterations and to a decrease in biodiversity. In the Galápagos Archipelago, one of the most damaging ant species is the tropical red fire ant, *Solenopsis geminata*. Our work showed that this species is completely dominant in disturbed areas and disrupts balance of local ant communities. To understand the life history traits, the population connectivity and the demographic history of the tropical fire ant, we characterised patterns of genetic variation in populations collected in Galápagos and South America using mitochondrial DNA sequences (CO1 and cytB) and nuclear microsatellite loci (12 independent and polymorphic microsatellite markers). Genotypes from more than 800 individuals (103 nests collected on 7 islands and in Guayaquil, main Ecuadorian harbour) revealed that genetic diversity is very low and that the inbreeding coefficient is extremely high. Results from a neighbour-joining reconstruction based on mitochondrial genes of 127 individuals from the Galápagos (N = 76), the Ecuadorian mainland (N = 2) and from 10 other countries in Latin America (N = 49) revealed 23 different haplotypes, of which only one was present in the Galápagos. This haplotype is also present in Guayaquil. Altogether, our results suggest that (i) tropical fire ant populations have undergone recent bottleneck, (ii) that a significant proportion of matings occurs between relatives, and (iii) that introduced populations of the Archipelago likely originated from Guayaquil. Our results are discussed in the light of the invasion risk of *S. geminata* in preserved areas of the Archipelago and across all continents, and in terms of potential threats to native fauna and economic impacts.

Allee effects through caste interaction feedback in ants

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Allee effects have important implications for many aspects of basic and applied ecology, in particular in endangered species conservation and in invasive species management. Allee effects are a result of positive impacts of aggregation of individuals and cooperation—driving factors of eusociality. Despite this, they have not been studied in eusocial insects. We studied Allee effects in the invasive Argentine ant (*Linepithema humile*). In the laboratory, we measured brood production and individual survival in experimental colonies that differed in initial numbers of queens and workers. While total productivity increased with increasing worker and queen abundances, *per capita* productivity decreased. However, this pattern of negative density disappeared when we accounted for the interaction between queen and worker abundances. Rather, we found evidence that queens and workers benefit from an increase in abundance of the alternative caste (e.g., *per capita* queen productivity increases with worker abundance). This suggests a potential positive feedback between worker and queen abundances, which may have contributed to the evolution of large colony sizes. Our study provides the first evidence for the presence of Allee effects in eusocial insects, and highlights the need to consider castes separately, along with inter-caste interactions, in the ecology of eusocial insects.

Group combats in ants: extending the Lanchester's laws

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Social animals often engage group combats where members of a same group cooperate during fight against conspecific or heterospecific opponents. In the simplest case, a battle is the result of a series of individual duels but in organized armies individuals from the more numerous group can cooperate in facing single opponents. Lanchester's laws have been used to model and interpret group combats in ants. It is known, however, that many of the assumptions on which these models are based do not easily apply to animal fights.
In this study we analysed the outcome of combats between groups of the invasive *Lasius neglectus*, and three native species (*Lasius paralienus*, *L. emarginatus* and *Crematogaster scutellaris*) were analysed. *Lasius neglectus* recently spread its range throughout Europe, causing serious concern for the effects on autochthonous ants. A preliminary set of observations and experiments were carried out to quantify: i) how individual fighting ability of the different species changes under different environmental conditions, ii) the time course of mortality; iii) how the willingness of individual ants to enter a combat is affected by previous experiences.
In the second part of the work, different group combat models were proposed. These started from classical Lanchester models, modified to include different constraints such as an upper limit to the number of individuals simultaneously involved in a combat, delayed lethal effects or changes in motivation to fight. Predictions from the models were then tested on the results of combats between opposing groups of native and invader ants species.

Dominance hierarchy among highly invasive ants

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Biological invasions and climate change are among the most problematic causes of biodiversity loss. Moreover, climate change could allow some invasive species to invade new environments by removing climatic barriers. This synergism between climate change and biological invasions is particularly problematic concerning invasive ants. Actually, five ant species are included among the 100 world worst alien species due to their dramatic impacts on many native species and ecosystems as a whole. Theoretical bioclimatic models predict overlapping potential habitat distributions for several invasive ants and consequently the potential of competitive interactions among them. The aim of our study was to investigate the dominance relationships among four of these invasive ants (*Linepithema humile, Pheidole megacephala, Wasmannia auropunctata, Lasius neglectus)* which may invade the same new areas. We observed interactions between pairs of species in single and group confrontations for all combination of the four species to construct behavioral dominance hierarchies. In addition, we investigated exploitative competition through colony-colony interactions. Our results show clear behavioral differences among the four species with a clear, linear dominance hierarchy among them. *W. auropunctata* was the most competitive species despite not systematically initiating aggressive bouts. Moreover, this species often feigned death in confrontations with the second more competitive species, *L. neglectus*. *P. megacephala* was the least competitive species. In areas where several of these species can become invasive, the knowledge of a potential exclusion of several of them by the most dominant is of paramount importance for conservation biology programs.

Hot stuff - Impacts of climate change on invasive ants

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Ants are among the most devastating invasive species and extremely difficult to control. They can have enormous impacts on native biodiversity and ecosystem functions. In addition, it is generally feared that climate change will favour invasive organisms, especially poikilotherms such as ants, by removing thermal barriers that currently prevent them from invading cooler regions In this study, we compare the potential habitat of 15 of the worst invasive ant species today and following climate change. The species' potential habitat has been estimated using ecological niche models (ENMs) based on 5 different modelling techniques, 3 Global Circulation Models and 2 CO2 emission scenarios, which have been combined in an ensemble forecast yielding a consensual model. Our results are unexpected and question the dogma of the synergism between climate change and invasive species. In brief, there was no unique trend for all of the 15 ant species, with several species increasing in invasive range, several others maintaining a similar range while several species even decreased in invasive range. In addition to this heterogeneity at a global scale, there was a considerable variation across regions for a given species, decreasing in some and increasing in others. Overall, our models indicate that the worst invasive species of today may not be the worst of tomorrow.

Effects of altitudinal gradient on ant community structure in Alpine ecosystems

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Since the mid-1990s, the consequences of global climate change for biodiversity are increasing their importance. Today, the main climatic parameters e. g., temperature and precipitation, are changing rapidly with drastic consequences for ecosystems, such as species extinction, species shift and changes in species composition. Altitudinal gradients are especially suitable to study these effects, thanks to the short distances between different ecosystems; indeed the altitudinal belt gradient represents climate variation over time. As a consequence of environmental changes, it is expected that species will respond in different ways and so community compositions will be disrupted. To this purpose, ants represent an ideal model because they show a measurable sensitivity to environmental changes, so their responses to different factors can be easily detected. During summer 2011, we led three samplings along an altitudinal gradient in one of the valleys of the Gran Paradiso National Park (north-western Italian Alps). Pitfall traps and food baits were paired to get information on numerical and behavioural dominance. According to “the Global Ants Collaboration” project, additional food baits were placed, using a standardized protocol in order to compare data collected all over the world. Results within altitudinal levels showed that species could be both numerically or behaviorally dominant or both. Results along the altitudinal gradient underlined that differences in ant community structure reflected different environmental conditions.

Assessing fragmentation and homogenization in ant communities of southern Mediterranean protected areas (Andalucia, Spain)

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Protected areas are central in biodiversity conservation. But in a decade of economies, efficiency to conserve natural patrimony is essential. In very urbanized countries (as is the case of Spain), protected areas might be more sensitive to homogeneization between regions and simplification of ecosystem. We have explored the conservation status of 32 protected areas in Andalucia (southern Spain) throughout different measures of biodiversity in ant communities. Our aim was to assess whether biodiversity could be affected by anthropogenic factors besides environmental characteristics, and whether biotic homogeneization could have increased similarity of ant communities between regions. A backward selection procedure was carried out to select which environmental and anthropogenic factors were responsible for determining high biodiversity indexes. Analysis of similarities (ANOSIM) and non-metric multi-dimensional scaling (nMDS) were used to assess similarities in ant communities among protected areas, habitat and landscape types. Biodiversity indexes were related to some spatial, climate and soil characteristics. Besides, and more importantly, distance to the limits of the protected area negatively affected richness, while less fragmented vegetation patches increased the number of endemic ant species. Vegetation cover affected positively richness and negatively the proportion of endemism. Analysis of similarities showed that ant communities were more similar within protected areas than among them, and community assemblages across different geological landscapes were different. Our study revealed that , in southern Spain, there is no homogeneization in ant communities between regions. However, it highlights 1) the importance to reduce the fragmentation of vegetation patches within protected areas; and 2) the importance of implementing a locally specific management of vegetation cover to conserve ant biodiversity.

The influence of urban green area management on ant communities

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According to the UN prospects, in 2050 the 70% of the worldwide population will live in urban areas. Global urbanization is leading to an intense transformation of natural ecosystems that are degraded, fragmented and more exposed to the risks of biodiversity loss. Since urbanization can not be stopped, making cities fit better with the objectives of sustainable development is a contemporary challenge. In this context, urban green areas are key elements and their creation, management and development should be carefully planned. In this work, we classified the urban green areas of Parma (a medium-sized town in Northern Italy) according to their size and management and then we studied their effects on ant biodiversity and community structure. Pitfall traps associated with food baits were used to achieve information on both numerical and behavioural dominance. The results and their interpretation show the sensitiveness of ants to the different management of the urban green areas. These data could be a useful source for the evaluation of current management plans and future developments of the town. In addition, this research demonstrates that ants are a good animal taxon for the study of urban ecology.

Soil properties weakly affect subterranean ant distribution at small spatial scale

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Physico-chemical soil properties are known to have an indirect effect on ground-dwelling ant assemblages at regional scale. By contrast, little is known on their effect at the scale of meter, at which direct interactions between subterranean ants and their environment occur.
We aimed to study the effect of soil properties on the small scale subterranean ant distribution in highly diverse tropical ant assemblages. We expected a differential response of ants to soil attributes, with particular species associated with a particular range of variables.
This study was conducted in two montane tropical forests 2km apart, Bombuscaro and Copalinga (Ecuador), sharing the same climate and botanical composition. In each site, we delineated a 100m transect and collected every meter a sample of soil (15×15×10 cm) and the associated ant fauna. The pH, organic matter content, electrical conductivity, humification degree, soil texture and slope were measured at each sampling point.
The soil attributes of the two sites were very contrasting. The ant assemblages shared only 12% of their 76 species. Ant diversity was higher in Bombuscaro (Fisher-α = 21.7 vs. 16 in Copalinga). Species richness was not correlated to any of the measured variables. Soil properties did not predict the occurrence of any ant species in Copalinga. In Bombuscaro, *Hypoponera 01RI* was correlated to lower pH values, and *Acropyga fuhrmanni* to higher clay content. *A. fuhrmanni* did not show correlation to this variable in Copalinga, where it is the dominant species (present in 48% of the samples). However, this species is presumably related to higher clay content, since Copalinga is much more clayey (36.7 ± 6% SD of clay in soil) than Bombuscaro (6.5 ± 2% SD).
Our results suggest a high species turnover at regional (2km) and at small (1m) scale, the latter being weakly explained by integrative soil parameters.

Ant community structure retains the past for a while... Ant community succession (Hymenoptera: Formicidae) on deciduous forest clear-cuts in Romania

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Ants are important components of forest ecosystems. Studied in combination with other focal taxa they might serve as useful biodescriptor tools with regards to the success of forest management processes. We studied ant community succession in deciduous forest clear-cuts in order to elucidate, how the composition and diversity of communities change in time, and whether these changes reproduce those occurring in the vegetation. Pitfall traps were applied at seven different aged forest sites (1-year and 2-years clear-cuts, 15-, 35-, 55-, 70- and 120- years old forests) as well as in two grasslands in Transylvania, Romania, for 10 days in three periods (spring, summer and autumn) in 2011. Additionally, the composition of vegetation was also analyzed. The most abundant forest ant species was *Myrmica ruginodis*, but *Temnothorax crassispinus* and *Stennama debile* were also present at almost every site. In the grasslands *Lasius niger*, *Formica cinerea*, *F. cunicularia* and *Camponotus piceus* were the most abundant ant species. As expected there was a clear difference between the ant communities of forests and grasslands both with regards to species composition and abundance. Plant communities showed a clear pattern concerning changes in species number which was decreasing during the aging. Surprisingly, ant species number did not show any major changes. Changes in vegetation structure showed a clear trend during succession, but ant community changes did not reflect the same trend, although clear differences among sites could be identified. Moreover, the structure of ant communities at the 1-year and 2-years old clear-cuts still resembled that of the old forests, thus retaining information on the existence of previous forests.

Phylogenetic analysis of the community structure of southern African termites

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Studying community assembly and structure of species allows insights into the fundamental mechanisms that dictate how species assemble and why communities are structured the way they are. In recent years there have been several improvements in this field, especially because phylogenetic data has been included into the analysis of community ecology and diversity, which has made it possible to explore theories about the influence of historical and ecological factors in structuring communities. There are two theories of coexistence that can apply to community structure. The niche theory, which states that species have to differ in their niches to coexist, and the neutral theory, which says that species are demographically equivalent and niche differences are not essential for coexistence. Here species distribution is due to random chance. We tested whether southern African termite communities are niche-assembled or if they are assembled randomly and which processes and traits are responsible for structuring communities (habitat filtering or competition). Because there are many cryptic termite species, barcoding was used for species identification. We could show that there is a regional species pool of 11 species in semiarid to arid regions of Namibia. No phylogenetic overdispersion or clustering could be identified, implying random assemblage of species. The analysis of 'spatial phylogenetic structure' showed that co-occurring species are distantly related to each other. This seemingly contradictory pattern can be explained by opposing ecological forces. Phylogenetic overdispersion generated by interspecific competition cancels out phylogenetic clustering generated by habitat filtering. This suggests that niches play an important role in termite community assembly in Namibia.

Close relationships between the yeast, *Saccharomyces cerevisiae*, and social wasps

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Saccharomyces cerevisiae is one of the most important model organisms and a good companion of human civilization. However, despite the wide usage of this microorganism in the last 9000 years, the existence of a complete life cycle in the wild has not been yet completely demonstrated. In this work we elucidate some still unclear phases of the *S. cerevisiae* ecological life cycle showing that social wasps living in temperate regions can be one of the environmental reservoirs of this microorganism. We provide evidence that social wasps overwintering as adults (*Vespa crabro* and *Polistes spp.*) act as vectors for harbouring yeast cells during winter, then spread them to the entire colony. We analysed microsatellites and sequences of a selected set of genomic loci able to recapitulate the yeast strains genomic profile, to compare the wasps isolates with a collection of more than 230 strains representing the worldwide yeast variation. The wasp strains do not form a separate sub-cluster but represent the overall observed yeast diversity of their geographic region thus representing an important environmental niche for the evolution of natural *S. cerevisiae* populations and the maintenance of their diversity. Yeast co-evolution with humans is probably the result of clonal selection resulting from a population boost during the biotransformation of fermented foods, followed by dispersal mediated by insect vectors.

Temporal changes of myrmecochory by the red ant *Myrmica rubra*

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Myrmecochory, is an insect-plant mutualism in which seeds develop a nutrient-rich appendage – an elaiosome- eaten by the ants. Plants also benefit from this association, by being dispersed by ant foragers that reject diaspores retrieved to the nest after feeding on seed elaiosomes. Our aim was to see 1°) whether the dynamics of seed harvesting changed over successive encounters with same plant species and 2°) whether changes in foraging responses can be related to the benefits gained by ants from elaiosome consumption. In this study we compared the foraging dynamics of the red ant *Myrmica rubra* when two well-known myrmecochorous species - *Viola odorata* and *Chelidonium majus*- were offered each week during 5 successive weeks. We focused on collective behaviours such as the foragers' flow, but also the number of retrieved seeds or their rejection rate out of the nest.

We found out a steep decrease of the number of seed harvested by ants already after 1 or at most 2 foraging bouts over the same seed species. On the 1st week, only 1 ± 4 *V. odorata* seeds (x + SD n=9) remained at the food source whereas most of the seeds (16 ± 4 seeds, n= 9) were left unretrieved by foragers on the 5th week. Such a decreased foraging was not due to satiation since it was reactivated when a new seed species or a small prey was offered. Furthermore, this decrease of the harvest process was not the same for the two plant species since it occurred later and to a lesser extent for *C. majus* seeds. This may be due to differences in the rate of elaiosome consumption since *C.majus* elaiosomes were more frequently eaten by foragers. We shall discuss about the actors and the mechanisms involved in this extinction process.

Interference of Social Insects on Human Affairs

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The urban environment represents the extreme form of human intervention on ecosystems. A city is like an island where natural schemes are transformed according to the dynamic of food fluxes and to the hygiene and health parameters of the different environments. In these places even the most harmless animals are perceived as elements of nuisance and contamination, giving rise to often exaggerated control actions that could be avoided with simple preventive measures and behavioral changes.
Objective: The aim of this work is to document how much and how social insects (especially wasps and ants) can enter in conflict with men, using a dynamic monitoring criteria and developing a tool capable to advise and support the community.
Methods: The estimation of the hazard related to social insects has been analyzed in the cities of Bologna and Florence and in the province of Venice, collecting all the requests attained in a pest control company from the municipalities, the local health units and private citizens. These references are concrete demands for action against unwanted animals and are all associated to specific reports that describe the technical details of the intervention performed.
Results: By means of the collected data it was possible to obtain a functional image of the analyzed territories, representing the “infestation status” by social insects at any given time and its evolution through the seasons, allowing to achieve the knowledge of the species and the factors that determine their abundance and to quantify their control costs.
Conclusion: With this analytic tool we intend to create a sort of forecasting calendar or a computerized alert system that provides feedback to the community on preventive measures and behaviors to adopt in case of events of acute infestation by insects which, although usually harmless, can sometimes be inappropriate, causing health, economic and psychological problems.

Influence of forage sources on *Andrena* species richness and abundance in meadows

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Solitary bees of the genus *Andrena* (Hymenoptera, Andrenidae) are important pollinators. Recently, *Andrena* species are facing severe diminishing of populations due to many factors, including habitat loss. The knowledge of forage sources represents a first step to avoid further declines of these bees. To achieve this aim, a survey was conducted in two meadows of north-eastern Italy. Field observations were weekly carried out from March to July 2007-2009 in two sites: A) a natural meadow ("magredo") and B) a semi-natural grassland with many synanthropic species, mostly due to past fertilization practices. *Andrena* specimens and plants, utilized as nectar and pollen sources, were counted and identified. In site A, 9 *Andrena* species were noted. The most abundant species was *Andrena hattorfiana*, representing more than 50% of all specimens observed in each year. In site B, 6 species were observed. Among these latter, *A. hattorfiana* was well represented in the first year, instead *A. flavipes* and *A. nitida* in the following years. In both sites *A*. *hattorfiana* was related to Dispacaceae: to *Knautia illyrica* as pollen source and to *Scabiosa* spp. for both pollen and nectar. *A. flavipes* mainly gathered pollen on Compositae: prevailingly on *Leontodon hispidus* in site A, instead on several species in site B. *A*. *nitida* intensively gathered nectar on *Thymus pulegioides*. *Andrena* species richness was higher in the natural meadow than in semi-natural grassland. Richness and abundance of *Andrena* species showed a discontinuous temporal trend in both sites. This pattern seems to be related to the different plant heritage and cover in each site over the years. Collected data suggest that to Compositae and Dipsacaceae belong the main important food sources for these bees. Even synanthropic plant species, as *Crepis* spp., *Erigeron annuus* and *Cichorium intybus* represent useful forage sources for *Andrena* species.

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Effects of overwintering temperature on the survival of garden ant *Lasius niger*

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Overwintering temperatures of ants can be higher in urban areas. In addition, overwintering temperatures may rise due to climate change. We studied whether the overwintering temperature affects the survival of the queens and whole colonies of the garden ant *Lasius niger*. In two consecutive years (2009, 2010) we collected colony founding mated queens (n=280) from the urban area of Turku (Finland) and divided them into two groups. Half of the queens overwintered in +7ºC and the others in +1ºC. After the overwintering period living and deceased queens were counted. In addition, we measured body fat content, body size and immune defence (encapsulation rate) of overwintered queens. With the same study design we studied the survival of 1-year old *L. niger* colonies (queen, workers & offspring). Overwintering in the lower temperature (+1ºC) and death of the mother queen decreased the survival of workers in the colony experiment. The survival of colony founding queens differed between years, but unlike in workers, the overwintering temperature did not affect the survival of queens; not in the colony experiment nor in the single queen experiment. All of the survived queens managed to produce their worker offspring. The body-size corrected amount of body fat of queens was higher in those overwintered in the lower temperature (less consumption). We did not detect differences in immune defence between the temperature treatment groups. *Lasius niger* has excellent dispersal ability and may not have strong local adaptation to certain overwintering temperatures. Thus it may be one of the species that do not suffer from changing climate. Its ability to tolerate wide overwintering temperature variations present in urban environments may explain its good success in urban areas.

The value of a holy forest for Nature conservation: ants in a refuge

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Holy forests were part of religious practice for generations. They are widespread from America to India, from Ivory Coast and Madagascar to Lebanon. Their surface is small but they were undisturbed sometimes for centuries. Our aim was to learn if such protected tiny forests could be beneficial for Nature protection. We sampled ants in Bar'am forest which remains the only dense *Quercus calliprinos* stand in Israel. Covering 100 ha, it was protected by the Maronite church (Westphal et al., 2009). Following its geographic situation and its particular ecosystem, it could be suitable for Palearctic animals. We surveyed the forest for ants using five different methods: pitfall traps in soil and on trees, leaf litter sampling and active search on ground and trees. These activities were processed along six transects of 200 meters each. In total we detected ants belonging to 37 species, 50% more species than found in the region in single studies (Martinez 2008a, b), and representing 15% of the total ant species in the country (Vonshak and Ionescu-Hirsch 2009). Among them 13 were officially recorded in the Upper Galilee for the first time. As expected, the majority of species had a Palearctic distribution. *Camponotus lateralis* was rediscovered in Israel: it was once caught by Forel in 1910. Two species were not surely definite and need a taxonomical revision: *Aphaenogaster* sp. near *epirotes* (Emery) and *Temnothorax* sp. near *affinis* Mayr 1855. Given that ant assemblages correlate with the composition of other components of invertebrate fauna (Majer, 1983; Andersen *et al.*, 2002), we understand that unmanaged tiny holy forest has an important role in regionl Nature conservation.

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The Italian School of Ants

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The School of Ants project is a study of the urban ant-fauna (Hymenoptera, Formicidae) carried out with the collaboration between scientists and citizens. It intends to involve teachers and students in collecting ants in schoolyards and backyards, using a standardized protocol in order to document the composition of ant communities that live in urban areas, particularly around homes and schools.
So this project has both a scientific aim and an educational one. On one hand, the collected samples can help to improve the knowledge about local biodiversity, native and introduced species in cities and to assess the effects of climate changes on urban areas; on the other hand, this study can teach to students the meaning and the importance of the word “biodiversity”, through the monitoring of the environment around them. The Myrmecology Lab (Dipartimento di Biologia Evolutiva e Funzionale, Università degli Studi di Parma) is the Italian partner of “the School of Ants” project, conceived by North Carolina State University researchers. Placing cookie baits outdoors, we sampled green spaces (lawns, gardens, woods) and paved places (asphalt, concrete, cobblestone) in some of primary and secondary schools in Parma (Italy). The results from this experience allowed to outline a preliminary picture of ant-fauna community structure in sampling areas, based on relative abundance and species richness data.

Session 5. Chemical ecology

Supercoloniality in the ant *Cataglyphis niger*

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Cataglyphis ants are one of the most characteristic insects of arid regions around the Mediterranean basin. In the present study, we wished to determine whether *Cataglyphis niger*, one of the commonest ant species in the Israeli coastal plain, is socially organized as supercolonies, using chemical and behavioral examinations, both in the field and the laboratory. Previous studies indicated that *Cataglyphis* workers are mutually aggressive, and that this is genetic-relatedness dependent. Workers are highly aggressive towards non-nestmates in comparison to nestmates. Nestmate recognition is carried out by sensing the cuticular hydrocarbon (CHC's) composition, which provides a nest specific chemical label shared by all nestmates. Since this species exhibits a limited nuptial flight close the ground, it is reasonable to assume that distances between nests also reflects genetic distances. The results of the behavioral experiments show that *C. niger* is organized in small clusters comprising 2-4 nests with no clear between-nests boundaries or territory. The workers exhibit low aggression towards nearby nests (presumably belonging to the same supercolony) and high aggression towards farther nests. It appears that the workers forage freely within the perimeter of associated (non-mutually aggressive) colonies. Two main social organizations can explain the results: 1. A central nest that contain the queens and a few satellite, queenless, nests in its surrounding (Polydomous). 2. A few nests that each contains queens and that interact in some way (Supercolony). Genetic and chemical investigations aiming at distinguishing between these possibilities are undertaken. Either way, this social organization probably enables *C. niger* to exhibit highly efficient foraging activity and largely exploit the habitat through focusing on colony expansion and vigorous intraspecific competition.

Nestmate recognition in the red wood ant *Formica polyctena* (Hymenoptera: Formicidae)

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Nestmate recognition plays an important role in social insects and cuticle lipids were shown to have a function as nest-specific recognition cues in most of the so far studied species including ants. The aim of this study was to identify in *Formica polyctena* the chemical substances with a function in nestmate recognition. For that purpose we performed bioassays with worker ants, chemical analyses via gas chromatography (GC) and GC coupled with electroantennography (GC-EAD) in combination with mass spectrometry (GC-MS). Furthermore ants were genotyped by the use of 7 microsatellites loci. Using Mantel tests we examined, whether aggression between worker ants increases with decreasing similarity of their odour bouquets and/or genetic distances.
In bioassays we were able to show, that cuticular hydrocarbons do play a role in nestmate recognition. Via GC-EAD we registered 23 active substances which could be identified by GC-MS as n-alkanes with chain length between C17 and C36, methyl-branched alkanes and 1 alkene. Bioassays with dead, odourless ants impregnated with surface extracts of ants or mixtures of the GC-EAD active synthetic linear and methyl branched alkanes in non-nestmate quantities indicated, that neither linear- nor methyl-branched alkanes alone elicit a high degree of aggression, while the combination of both released aggressive behaviour of the tested ants comparable to tests with non-nestmate odour. A discriminant function analysis performed with EAD-active compounds showed that odour bouquets of workers from different nests differed significantly and revealed more similarities among polydomous nests compared to non-polydomous nests. Mantel tests revealed a weak correlation between the frequency of aggressive behaviour in interactions of worker ants and their genotypes or their odour bouquets. The missing correlation we found between genetic similarity and similarity of odour bouquets may be explained by recognition cues of an exogenic origin.

Nestmate recognition code: Variation in relative proportions of hydrocarbons may open social wasp colonies to aliens

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We know much of how social insects recognize nestmates. We know that social insects use a chemical code composed of epicuticular hydrocarbons, we know that branched hydrocarbons may convey more information than unbranched ones; we know that hydrocarbon quantity matters. However, we still often do not know how the code works. Facultative social parasites, such as the free-living social wasps *Polistes nimphus,* overmark the host colony odor with their own and therefore change host colony odors. We used nest usurpation between *P. nimphus* and *P. dominulus* as a means to alter colony odor and identify the hydrocarbons important in the nestmate recognition code. The colony odor in these two species is very similar because the two species have chemical signature that differ only in their relative proportions of hydrocarbons. In usurped nests, we tested how host workers responded to alien conspecific wasps and to alien *P. nimphus* wasps. Behavioral and chemical analyses showed that facultative social parasites altered the host colony odors by making small changes in the relative proportions of four branched hydrocarbons and an alkene. These small changes had a large effect on how workers responded to aliens. Host workers were tolerant towards any alien *P. nimphus* wasp as if they were unable to distinguish their individual odor variations. However, host workers were not tolerant towards conspecific alien wasps, indicating that they were still able to discriminate conspecific aliens. These results document that some hydrocarbons may play key roles in wasp recognition codes and that the variation in the relative proportions of hydrocarbons is an important property in the recognition code.

A multilevel and comparative approach to study nestmate recognition in a sympatric species complex of Neotropical ants

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The ecological success of social insects' colonies lies in their ability to prevent the exploitation of colony resources by competitors and parasites. Nestmate recognition, i.e. the process by which individuals discriminate colony members from strangers, is therefore of crucial importance in the maintaining of the society's cohesion. The effectiveness of this recognition system largely depends on reliable recognition cues, and cuticular hydrocarbons have been shown to play a central role in the decision to either accept or reject an individual. Furthermore, numerous species display various responses towards different foreign colonies, suggesting that nestmate recognition may involve context-dependant and complex cognitive processes. However, the underlying proximate mechanisms remain largely unknown, particularly in an interspecific context. Using *Pachycondyla apicalis* ants, a complex of closely related sympatric species, we studied how nestmate recognition and dominance relationships were influenced by spatial, chemical and genetic distances between colonies, from the intracolonial to the interspecific level. We performed dyadic encounters between individuals originating from nests at various spatial distances (sympatric neighbours, sympatric non-neighbours, and allopatric nests), and at different interaction levels (intracolonial, intraspecific, and interspecific). We assessed the chemical proximity of all studied colonies by the composition of their cuticular hydrocarbon profiles with GC-MS SPME. Finally, genetic distances between nests were investigated using microsatellite loci and mitochondrial cytochrome b. The behavioural, chemical and genetic correlates allow a better understanding of the proximate mechanisms at the basis of nestmate recognition. We discuss the influence of cuticular compounds on the determinism of behaviour, and their potential implication on the divergence processes of these closely related species.

Pre-imaginal experience and nestmate recognition in the ant *Aphaenogaster senilis*

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The social homeostasis of insect societies depends on the ability to discriminate between nestmates and non-nestmates. According to a label-template matching model, the chemical label of an encountered individual (its odour profile) is compared to the template of the evaluator (a neural representation of the profile). Several studies suggested that ants learn the template during the first days of adult life but there is some evidence that pre-imaginal learning may occur during the larval stage. The discrimination process typically results in the occurrence of aggressive interactions among adult workers from different colonies, whereas immature offspring can usually be transferred between alien nests. In this study we exploited this feature to investigate whether in the ant *Aphaenogaster senilis* there is a sensitive period for pre-imaginal learning during the larval stage that will influence nestmate recognition at the adult stage. Larvae of different developmental stages were transferred as a group into a non-nestmate "recipient colony" whose own brood had been eliminated. The adoptees were left to develop in the "recipient colonies" and later retransferred back into their "mother colonies" and left to become adults. After ten days of adult life, the aggressive response of these experimental ants, versus "mother colony" individuals, "recipient colony" individuals and alien individuals, was investigated. The results show that nestmate recognition is affected by pre-imaginal experience depending on the age of the larvae when adopted. Ants transferred into a recipient colony as first-instar larvae are less aggressive towards individuals from this familiar colony than towards alien individuals; but this is not the case when ants were introduced in the recipient colony as last-instar larvae. Chemical analyses of cuticular hydrocarbons of each colony revealed that the pre-imaginal learning process is more efficient when the colonies' chemical profiles (mother and recipient) are more similar.

Mixed maternal, paternal and environmental effects on cuticular substances in *Cardiocondyla obscurior*

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Chemical cues are essential in social insect communication. Cuticular hydrocarbons have been found to function in a wide array of interactions, from caste and fertility identification to nestmate recognition. Empirical data suggests that the environment affects colony-specific odours to a large degree. Van Zweden et al (2010, Evol. Biol. 23: 1498) in addition showed that a genetic component is responsible for shaping the profiles of a *Formica* species using cross-foster experiments. I will present preliminary results testing the hypothesis that both maternal as well as paternal genetic background affects colony odor. We used intra- and interpopulation crosses of queens and males from a Japanese and a Brazilian population that have been cultered in the lab under similar conditions for more than one year. The resulting F1 workers were raised by either Japanese or Brazilian nurse workers. Shortly before eclosion of the F1 workers we removed the nurse workers, thus nurses could only 'prime' F1 brood during development. We extracted cuticular substances and compared nurse workers and F1 workers. This design allows to simultaniously test direct influence by the nurses (environment) and maternal as well as paternal influence. The results show that a complex interplay of exterior environment, age, direct transmission and heritability shape the profiles of *C. obscurior*. Though direct contact between adult F1 workers and nurses was prohibited (only at the larval stage) the nurses had the strongest effect on colony odor. In addition both maternal and paternal background strongly affect the relative proportions of particular substances.

Nest wax signals and their influence on worker reproduction in *Bombus terrestris*

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The organization of social life in insects is based on a highly efficient information transfer between colony members. When encountering a conspecific individual, females in many social insect species are able to identify its caste membership, sex, kin, nestmate and fertility status by means of cuticular lipids. A growing number of studies show that these substances can also have a communication function beyond the recognition of individuals. In this study we examined the signaling means of cuticular lipids present in the nest wax and their effect on social behavior in *Bombus terrestris* colonies.
Our chemical analyses of wax extracts showed that wax from bumblebee colonies contained the same pattern of substances - mainly hydrocarbons, fatty acids and esters - that functions as fertility signal on the cuticle surface of female bumblebees (Sramkova et al. 2008). The chemical patterns of nest wax lipids differed between individual colonies, developmental stages of the colony and various nest structures. As in honeybees, the pronounced pattern differences due to colony identity allowed bumblebees to discriminate between wax from their own and foreign nests in olfactometer bioassays. However, not only colony identity but also colony age had a strong effect on wax profile variations. Interestingly, similar changes of the same subset of substances occurred in all colonies with increasing colony age. In behavioral assays we found, that these changes in colony scent influenced the decision of workers to reproduce. Worker groups started to compete for reproduction despite the presence of a queen when facing wax from a later stage of colony development.
From our observations that lipid profiles in wax can provide a multitude of information and directly affect worker behavior, we suggest that wax scent might play a decisive role in the social communication of bumblebees. (DFG project Ayasse 12/3-1)

*Sramkova A. et al. 1998 Naturwissenschaften 95(6): 515-522.

The effect of precocene, a JH biosynthesis inhibitor, on the establishment of reproductive dominance in bumblebees

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Precocene is known to block JH biosynthesis and atrophy of the corpora allata (CA) in many insects, but its effect in Hymenopteran has been only sporadically reported. In this study we tested how precocene I affects reproductive dominance among *Bombus terrestris* workers. We kept groups comprising 3 callow, same-size workers for 7-days, each randomly assigned to one of the following treatments (1) feeding of 1 mg/worker precocene in sugar-water; (2) feeding precocene and topically applying 100µg JH-III dissolved in 2.5µl Dimethylformamide; (3) feeding precocene in addition to topically applied 2.5µl Dimethylformamide; (4) untreated control groups. The precocene solution was administered upon group establishment and was usually consumed within 24 hours, after which the workers were fed sugar-water. Pollen was provided throughout the experiment. Treatments with JH or solvent were made on the second day, and behavioral observations were performed for 30 minutes daily during days 3-4, while monitoring aggressive behavior. By day 7, a hemolymph sample was taken for JH determination and the workers were dissected to measure their terminal oocytes and extract Dufour glands secretion. QL workers show elevated hemolymph JH, and developed ovaries accompanied by the disappearance of Dufour's gland ester sterility signal. Precosene inhibited both JH level augmentation, as determined by combined GC/MS, and ovarian development in such workers. Precosene treated workers were also significantly less aggressive and possessed higher amounts of the ester-sterility signal compared to control groups. Treatment with JH alleviated only partially the effect of precocene in that treated workers showed high reproductive development but not any increase in aggressive behavior. This suggests that other, CA-dependent substances may be involved in the establishment of reproductive dominance. The response of *B. terrestris* to chemical allatectomy opens new avenues for exploring the role of JH and possible other CA products in reproductive competition.

“Smells Like Queen Spirit“

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Queen pheromones are well studied in social insects and a keystone of reproductive division of labour. The differentiation of castes in termites is generally induced by differential gene expression and the recognition of individual castes is mainly mediated by cuticular hydrocarbons (CHC) which organise colony behaviour. This means that queens and workers have different CHC profiles that maintain the reproductive monopoly of the queen which is important in wood-dwelling termites: here, workers are totipotent to develop into reproductives either via several moults into winged sexuals that leave the nest and found new colonies or via a single moult into neotenic reproductives when the current reproductives fall ill/die.
To link the molecular level with behaviour we focused our studies on *Neofem4* (neotenic female specific gene), a cytochrome P450 gene (CYP4) which is overexpressed in queens of *Cryptotermes secundus*. This gene family is known to be involved in the production of CHCs in cockroaches. Using RNA interference we silenced *Neofem4* in queens and analysed their CHC profiles. We found that the queens’ CHC profiles changed to a worker-like profile with less compounds. The change of the profiles was in line with an increase of butting behaviour by workers which is typical for queenless colonies. Thus we conclude that *Neofem4* plays a major role in the recognition of the queen by the colony and is necessary as a dominance signal.

Explosive backpacks in old termite workers

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We send our young men to war, ants send their old ladies [1]. This metaphor illustrates a long-known feature of insect society ergonomics, i.e. that older workers generally assume a larger share of the riskiest tasks, such as foraging or defence [2]. More precisely, it is remaining fitness rather than absolute age which determines the cost of engaging in risky activities [3-5]. Among fully sterile castes of social insects, suicidal defensive behaviour may be favoured, if benefits to the colony overweigh the cost of losing one of its members. Here we show that, as workers of the termite *Neocapritermes taracua* age and their food-collecting ability declines, they develop a unique two-component suicidal defensive apparatus consisting of copper-containing blue protein crystals, stored in an external pouch over the metathorax, and intra-abdominal salivary gland vesicles. During fight, the dorsal abdominal wall ruptures and the crystals react with the labial gland secretion to produce a toxic and sticky droplet; both the amount of defensive substances and the readiness to explode increase with workers' age. Our findings represent an excellent example of age-dependent transition to risky tasks in social insects, corroborating the predictions of kin selection theory [4]. At the same time, we reveal an intricate and spectacular suicidal defensive mechanism, without any equivalent in social insects.

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Recruitment behaviour and communication in *Partamona orizabaensis*

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Social insects have evolved communication systems that allow them to recruit their nest mates to food sources. The foragers of several species of stingless bees deposit scent trails consisting of pheromones on solid substrates to guide their nest mates to food sources. More than fifty years ago, Kerr (1969) stated that stingless bees of the genus *Partamona* create aerial odour trails to recruit additional workers to resources. He postulated that the foragers release their pheromones during flight and lead the naive bees in large groups to the food source. However, experimental proof for this assumption is lacking. We, therefore, studied the recruitment behaviour and communication mechanisms in the species *Partamona orizabaensis*.
During field experiments, newly recruited bees had to choose between two feeders, one visited by experienced foragers (recruitment feeder) and an unvisited control feeder. The recruits always preferred the recruitment feeder over the control feeder, indicating that an efficient recruitment to the known feeding site took place. However, we never observed scent trail marking between the feeder and the nest, as known for other species (e.g., *Trigona*).
In additional bioassays, we recorded the bees' choice behaviour towards feeders baited with labial- and mandibular gland extracts. The bees did not prefer any of the gland extracts. Rather, the compounds extracted from mandibular glands had a clear deterrent effect.
Chemical analyses revealed that the labial gland secretion is predominately composed of alkenes, whereas the mandibular gland secretion also contains alcohols and esters. This is interesting because esters are known to serve as recruitment pheromones in trail laying stingless bee species. Whether these compounds are released by *Partamona* foragers during flight in order to guide recruits to food sources, as stated by Kerr (1969), needs to be clarified in future studies. (We acknowledge funding by DAAD)

Kerr W. E. 1969. Evol. Biol. 3: 119-175

Choosing an aphid partner: a matter of taste and smell

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Honeydew is the keystone upon which ants and aphids build their mutualistic relationship. We have investigated how sugar and volatile compounds from honeydew are involved in the discovery, the recognition and the exploitation of aphid colonies by the black garden ant *Lasius niger*. In addition to semiochemicals produced by aphids, honeydew volatile compounds are used by ant scouts to orient themselves and distantly recognize myrmecophilous species. Once discovered, aphid colonies producing sugars which are the most beneficial to the ants are preferentially tended. In this respect, the ways each sugar acts upon the feeding behavior of ant foragers and triggers the laying of a recruitment trail are essential to understand how their collective exploitation of aphid colonies proceeds and why mutualistic interactions between ants and aphids are maintained.
Sensitivity of ant scouts to honeydew sugars was also investigated. Dose-response curves revealed between-sugar differences with foragers being very sensitive even to small amounts of melezitose, a sugar specifically produced by aphid colonies. We discuss about the relevance of honeydew cues used by ants in the selection of sugary resources, the recognition of their honeydew-producing partners as well as in the assessment of size and nutritive value of exploited aphid colonies.

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Chemical structure of odorants and perceptual similarity in ants

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Animals are immersed in a chemical world consisting of mixtures of many compounds rather than of single substances, and they constantly face the challenge of extracting relevant information out of this chemical landscape. The ability to discriminate among different stimuli is extremely important, together with the ability to generalise, which allows treating different but similar stimuli as equivalent. We measured perceptual similarity of different odours by conditioning individual carpenter ants (*Camponotus aethiops*) to one odour, and testing their response to another, structurally different odour. We show that asymmetry in generalisation, where ants generalise from odour 'A' to 'B', but not from 'B' to 'A', is not only dependent on chain length, but also on functional group of chemical substances. By conditioning individual ants to a binary mixture, and testing their reaction to the individual components of the mixture, we show that overshadowing, where only parts of a mixture are learned better than others, is rare. Generalisation is not only dependent on the structural similarity of odorants, but their functional value might play a crucial role. Our results are important to understand how ants perceive and make sense of the complex chemical world around them, and provide a solid basis to elucidate the neural mechanisms behind asymmetry in generalisation and perceptual similarity in general.

Differential coding of floral and pheromonal odors by two olfactory subsystems in the honeybee brain

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Sensory systems use parallel processing to extract and process different features of environmental stimuli. Parallel processing has been studied in the auditory, visual and somatosensory systems, but research in the olfactory modality has shown little progress. An invertebrate model like the honeybee is well-suited for such research, as it provides relative neuronal simplicity with good experimental access to the brain and robust behavioural paradigms. Strikingly, the honey bee brain contains a dual olfactory system, with a clear dichotomy from the periphery up to higher-order centres, subtended by two main neuronal tracts (median and lateral Antenno-Protocerebral Tract, m-APT and l-APT). The function of this dual system is utterly unknown, and attributes like odour quality and odour quantity may be separately encoded in these subsystems. We have thus started a thorough functional study of olfactory coding in both subsystems, using *in vivo* calcium imaging to reveal neuronal activity. As one of the subsystems (m-APT) has never been imaged before, a novel imaging preparation was developed and responses to floral and pheromonal odorants at different concentrations were compared in both subsystems. Our data show some redundancy of olfactory coding for general odours in the two subsystems, but unravel striking specificities of each system for encoding queen, brood and worker pheromones. Our current experiments aim to understand neural processing at higher levels of both pathways.

Differences in the protein pattern of male accessory glands between winged and wingless males of two *Cardiocondyla* species

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In the ant species *Cardiocondyla obscurior* and *C. tjibodana*, two different types of males occur. “Normal”, winged males leave the maternal nest several days after eclosion to mate outside, whereas wingless, “ergatoid” males stay life-long inside the nest. In *C. obscurior*, the ergatoid males are produced regularly and winged males occur only when environmental conditions deteriorate. Contrary, in *C. tjibodana*, ergatoid males are reared only occasionally, but winged males are usually present in the colony. It has been shown that in *C. obscurior* queens live longer after mating with a winged male than after mating with an ergatoid male (Schrempf A. et al. 2005. Current Biology 15: 267-270). As accessory gland proteins are known to have a dramatic influence on the lifespan of female insects (Chapman T. et al. 1995. Nature 373: 241-244), we determined the variation of the protein patterns of male accessory glands both between the types of males within species as well as between species. To do so, we conducted 2D-gelelectrophoresis with subsequent silver-staining. The spots were analyzed and compared using the software progenesis.

Do honeybees recognize eggs by peptides?

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In many social insects, workers recognize the eggs of their own queen(s) and discriminate against any other eggs. This has been first demonstrated in the Western Honeybee (*Apis mellifera* L.), where workers selectively remove eggs laid by other workers (Ratnieks & Visscher 1989). This behaviour has been called 'worker policing'. Since queen laid eggs are usually not removed, honeybee workers have to be able to recognize the origin of a given egg. It has been hypothesized that queens mark their eggs with an egg marking pheromone that cannot be faked by workers. Indeed, in the ant *Camponotus floridanus*, queen laid eggs and queens have a similar chemical profile of cuticular hydrocarbons that serve as a queen signal of the queen's presence (Endler et al. 2004). However, in honeybees it remains elusive which cues or signals are used by workers to recognize queen laid and worker laid eggs, respectively. There are no conspicuous differences in the physical appearance, nor did chemical analyses reveal a single compound or compound class that might account for the discriminatory abilities of honeybee workers (Martin et al. 2005). Here, we suggest that peptides might have been overlooked in the search for the egg-marking signal in honeybees. We extracted peptides from the egg surfaces of queen laid and worker laid eggs, respectively, and analysed these extracts with two types of mass spectrometers. We identified several peptides, including antimicrobial peptides and venom components. We will discuss the potential of this finding for the role of peptides in the recognition and discrimination of honey bee eggs in worker policing, as well as the hypothesized function of peptides in the protection of eggs.

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Biochemical crowdsourcing through oral fluid-exchange in ant colonies

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Communication is essential in high-functioning social groups, from social insects to humans. How do ants bring about colony-wide change without language or top-down control? While ants are traditionally thought to communicate mostly through pheromones, we are testing whether trophallaxis – a method of mouth-to-mouth liquid transfer – may also be an important pathway for communication enabling a form of chemical crowdsourcing. Given the power of trophallaxis to rapidly distribute liquids throughout the colony, trophallaxis would provide an excellent means of information transfer, especially for compounds unstable outside the body. Socially exchanged fluids, *e.g*. seminal fluids, often carry ancillary information that alters behavior. To date, the contents of trophallaxis fluids have not been thoroughly analyzed and thus the precise function(s) of this striking behavior remain unknown. Using nano-liquid chromatography tandem mass spectrometry, we have biochemically analysed the protein components passed between individual ants during trophallaxis. In addition to the anticipated insect gut proteins, we have also found proteins potentially involved in the hormonal regulation of social insect behavioral maturation – the age-related transition from nurse to forager. A number of proteins passed between nestmates appear to vary depending on the social caste of the donor and have high sequence similarity to well-known insect hormonal regulators, suggesting that by passing regulatory molecules to one another, nestmates might influence one another's behavioral maturation. These biochemical studies are being paired with a highly quantitative, barcode-based ant tracking system to measure the behavioral causes and effects of trophallaxis and the long-term behavioral effects of candidate proteins and their inhibitors.

Recognition of nests and nestmates in the African stingless bee *Hypotrigona gribodoi*

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Behavioural studies of the highly eusocial stingless bees (Meliponini) have been almost exclusively restricted to neotropical species. Little is known about the behaviour of Old World meliponines. The nests of the African stingless bee species *Hypotrigona gribodoi* are often aggregated in space, the nest entrances and surroundings appearing visually quite similar and close to each other. We therefore studied the roles of visual and chemical cues in the orientation behaviour of foragers returning to their nest and the chemical cues used to discriminate nestmates from non-nestmates at the nest entrance. Experiments with 25 different nests have been conducted in the Soutpansberg, South Africa. After different manipulations at the entrance tube (e. g. exchange of entrance tube, use of fake entrances) bees need a significant longer time for homing compared to control experiments. The results show that both, visual and olfactory cues are used by homing foragers. *Hypotrigona gribodoi* uses the undesirable-absent recognition system to discriminate their nest entrance from others. Furthermore *Hypotrigona gribodoi* is able to differentiate their own from foreign wax. The Bees are able to discriminate nestmates that acquired a foreign wax odour from those that acquired their own wax odour. Agonistic behaviour in arena-experiments occurs significant more often if nestmates acquired a foreign wax odour compared to own wax odour. This indicates undesirable-absent recognition system as well. These experiments reveal that *Hypotrigona gribodoi* recognises alien odour cues and is thus detained to enter an alien nest and identifies friend and foe.

Hymenopteran birth control: ants and wasps share conserved class of sterility-enforcing queen pheromones

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In many insect societies, the queen inhibits the reproduction of her daughter workers through the emission of unique fertility-signalling chemicals, but for most social insect species the identity of such queen pheromones remains completely unknown. In this study, we perform bioassays in which we add synthetic queen-specific hydrocarbons (i.e. cuticular hydrocarbons that are especially abundant on the cuticle and eggs of queens relative to those of workers [1]) to queenless nests of the common wasp, *Vespula vulgaris*. Here, we report the identification of a sterility-inducing queen pheromone in the common wasp, and show that the active compound is a long-chain alkane, *n*-nonacosane, that is structurally similar to a queen pheromone recently identified in ants [2,3]. A comparative analysis of the identity of queen-specific or fertility-linked compounds in 55 species of eusocial ants, bees, wasps, and termites further shows that ancestrally, ants and wasps both likely used simple saturated hydrocarbons as sterility-enforcing queen pheromones. This is remarkable, given that these two groups are separated by ca. 150 million years of independent evolution [4], and suggests that eusociality in both groups is regulated by a highly conserved signalling system that originated in a common solitary ancestor.

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Comparison of Cuticular Hydrocarbons Profiles Among Nature and Produced Workers and Queens in the Stingless Bee *Frieseomelitta varia* (Hymenoptera, Apinae, Meliponini) and Acceptance of the New Queen

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The ability to discriminate nestmates from non-nestmates is critical to maintenance the integrity of social insect colonies. These compounds are important chemicals involved in processes such as recognition of nestmates, castes, and genders (Blomquist *et al*, 1998). The stingless bees have singly mated queens, therefore, are expected to have a higher chemical homogeneity between individuals. According our technique developed for *in vitro* production of stingless bee queens, only few produced queens were accepted by workers. For that reason, we intend to characterize the variation of the chemical profiles among natural and produced workers and queens of *Frieseomelitta varia*. The produced individuals were made *in vitro* transferring the larvae from natural colonies to acrylic plates and keeping under controlled conditions of temperature and humidity. Natural workers (NW, n= 9) and virgin queens (NQ, n= 7) and produced workers (PW, N=9) and virgin queens (PQ, N=10) were collected in small glass vials and killed by freezing. The cuticle lipids were extracted by immersion of the whole body in hexane (0.5 ml) for 1 min. 1 µl of each extract was injected and analyzed in a gas chromatograph and mass spectrometry system (SHIMADZU, model GCMS-QP2010). The compounds were identified and the comparison between groups was made by multivariate statistical analyses. The Mahalanobis distances showed that the produced queens have a cuticular profile similar to the natural virgin queens (0.401) when we compared to the profiles of the other two groups (PW= 3.62, NW=742.33). The discriminant analysis showed that 8 individuals of PQ were correctly allocated into their group and 2 individuals into the NQ group ($p<0.5$), according their chemical profile. We believe that the chemical similarities and dissimilarities of cuticular hydrocarbon profiles can induce the behaviour of *F. varia* workers during the acceptance process of new virgin queens.

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Behavioural interactions between larvae and workers in ants: the role of larval odours in stress situation

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In eusocial insects, larvae are entirely dependent on adult workers who take care of them and supply them with food. However, as Schneirla (1957) suggested in the brood stimulation theory, larvae are far from being passive elements of the colony and may act as modulators of the adult behaviours, especially foraging behaviours. Our recent work in *Ectatomma tuberculatum* ant (Ectatomminae) showed that starved larvae display frequent movements and probably emit chemical odours that trigger nursing behaviours from the workers. Both tactile and chemical stimulations can be used as communication signals regulating adult behaviours. We test here the hypothesis that larval odours alone can modify workers' responses. We then compare the interactions between larvae and workers in experimental colonies during two phases, i.e. when a purified air flux is flowed (control phase) and when a purified air flux with odours from starved larvae is flowed (experimental phase). The individual foraging characteristics and brood care frequency are noted and compared in both phases. We expected to disentangle between chemical and tactile larval stimuli in the complex system of social communication in ants.

Schneirla T. C. 1957. Proceedings of the American Philosophical Society 101: 106-133

How colony nutritional state affects individual response thresholds to larvae in ants

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Division of labour is considered as one of the core adaptations leading to the success of the social insects. The response threshold models, among other models, explain task allocation amongst workers by the existence of differential individual thresholds. They postulate that each task is associated to a specific graduated stimulus and that each worker possesses an internal threshold which determines its probability to perceive the stimulus and to respond to it. When the intensity of the stimulus exceeds its internal threshold, the individual performs the specific behavioural task. At colony level, the heterogeneity of the response thresholds between workers constitutes a simple mechanism providing a variety of behavioural patterns. At individual level, the value of the response thresholds depends on internal and external factors. Among them, the nutritional factors may influence the capacity to react to task-related stimuli through physiological processes. To test this hypothesis, we study the effect of the workers' nutritional state on their ability to respond to larval stimulation associated with specific tasks in the ant, *Ectatomma tuberculatum*. We manipulate the quantity and quality of food supply to experimental colonies and measure the ability of nurses, domestics and foragers to respond to larval odours. Two olfactometry tests with living larvae were used as bioassays. Results are discussed in the context of the response threshold models and their implication in the individual decision making process and the regulation of social interactions.

Silenced by the regime: honeybee workers exposed to virgin queens develop ovaries but fail to advertise fertility.

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Queen mating status in social insects is a matter of crucial importance for workers because of its influence on the queen's productivity and consequently their fitness. Behavioural and physiological reactions of workers to the queens mating status have been studied as a proxy to mechanisms maintaining insect sociality. Here we show that unmated honeybee queens have considerably impaired capacity to trigger worker sterility in comparison to mated (and thus more productive) queens. Together with this it is shown that honeybee workers exposed to unmated queens despite being active reproductively display an impaired ability to advertise their fertility compared to queenless workers. These findings suggest that reproductive development and production of fertility signals are differentially regulated and differently influenced by the queen's presence.

Chemical correlates of male fighting in an ant species with a male diphenism

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Like other species of the ant genus *Cardiocondyla*, *C. wroughtonii* shows a peculiar male diphenism. Wingless males remain in their maternal nests and mate with the present virgin queens, while winged males attempt to mate first with queens from their natal nests and later disperse to mate with queens from other colonies. Wingless males are very aggressive towards other wingless males and consequently only a single wingless male exists per colony. Wingless males do not behave aggressively towards their winged rivals. In the sibling species *C. obscurior* winged males appear to be protected from wingless males by mimicking the cuticular hydrocarbon profile of virgin queens (Cremer S. et al. 2002. Nature. 419: 897). We performed gas chromatographic analyses of young and old queens, winged males, wingless males, and workers. Young winged males and winged female sexuals differed from their nestmates in the proportions of a few branched alkanes and alkenes. However, the distribution of other peaks does not support chemical mimicry. For example, wingless males were characterized by a particular substance absent from all other nestmates, which might allow adult wingless males to recognize and kill young wingless rivals.

Do environmental factors influence social organization traits in *Reticulitermes* termites?

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Termites are characterized by a caste system where each individual is specialized in certain tasks. Reticulitermes is a good model because of the complexity and the flexibility of its caste system. The caste determination is based on genetics factors as well as environmental factors and social interactions. Hydrocarbons (CHCs) play a key role in social insects' communication by allowing recognition between colony members and consequently permitting social cohesion.
We studied the impact of the presence of CHCs in near environment on social organization traits of two Reticulitermes termites: *R. grassei* and *R. flavipes*. For that purpose, we focused on caste ratio, paper consumption and survival rate. First of all we extracted CHCs from: (i) an ant (Lasius niger), (ii) workers and (iii) soldiers of the two termite species to simulate predators as well as inter and intraspecific competitions. Solvent only was used as control. CHCs were transferred on a filter paper disk and added into termite nests with nutritive filter paper disks. 350 workers per nest were reared during 2 months in those conditions. We changed the odor disk once a month and food disks once a week. Every week we surveyed caste ratio and disk consumption. After 2 months with CHCs treatments, we studied behaviour of workers in a competition test. Ourresults show that the presence of CHCs extracts in near environment did not change caste ratio but did influence their behaviour by increasing survival rate.

Population analysis of *Crematogaster scutellaris* colonies: combining behavioural, genetic and chemical informations.

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Crematogaster scutellaris is a common ant throughout the Mediterranean basin, which occupies a dominant position within competition hierarchies. Previous studies showed a clustered distribution of nests of this species and suggested a polydomous arrangement of colonies. The relationships among different colonies were investigated combining aggression experiments, genetic analysis of population structure, and chemical analysis of cuticular hydrocarbons. The study was carried out in an olive orchard near Florence (northern Tuscany, Italy), where the only available nest sites were represented by olive trees. All the nests in an area of 2.5 hectares were mapped. Nests showed a clumped distribution with defined spatial clusters (defining a cluster as a group of nests not separated by an empty space in between). A subsample of 10 of such clusters (for a total of 34 nests) were randomly chosen for subsequent analyses. Six workers were randomly collected from each nest (n=204) and genotyped using 9 microsatellite loci, specifically designed for *C. scutellaris*. Six additional workers were also collected for gas chromatography/mass spectrometry chemical analysis. A total of 91 aggression tests among ants from different nests were also carried out in the field. Results showed that the 34 nests belonged to 14 totally unrelated colonies and confirmed the polydomous structure of colonies. Aggression was not related to genetic distance, but was inversely correlated with cuticular hydrocarbon similarity among nests (aggression was more likely to occur between nests sharing similar hydrocarbon profiles).

Does Cuticular Hydrocarbon composition explains acceptance and rejection behaviour in the honeybee?

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The importance of Cuticular Hydrocarbons (CHs) as colony recognition cues in social insects is supported by two main kinds of experimental results:
-Higher similarity in composition between individuals belonging to the same colony than between those belonging to different colonies
-Rejection of nestmates when their CH profile is altered through application of CHs extracted from other colonies, or through application of single CH components
Most recognition experiments are conducted under unnatural or semi-natural condition that requires manipulation of individuals or colonies. Here we report the results of experiments on honeybees (*Apis mellifera*) that investigated if CH composition could explain acceptance or rejection behaviour of conspecific under totally natural conditions.
Interaction between guards and forager bees landing on the nest were observed in three different colonies belonging to the same apiary. Guard behaviour towards each landing bee was scored as "acceptance" or "rejection" and then each landed bee (N=140) was captured by the investigator. These individuals were analysed for their CHs through GC-MS and genotyped through the analyses of four different microsatellite loci (A29; Ap33; A76; A107) previously analysed from a sample of nurse bees from each study colony (N=138). On the basis of their genotype they were scored as nestmates (NM; 55,7%) or aliens (A, 44.3%) with respect to the colony where they landed. By comparing behaviour and genotype we found that colonies showed limited differences in the percentage of bees correctly recognised (87%, 84% and 83%), but while one of the colony performed several acceptance errors by accepting A (23% against 12% and 6%), the others made several rejection errors, by rejecting NM (19% and 23% against 3%).
Multivariate discriminant analysis on CH composition of NM accepted on their own nest (67 out of 78) showed a clear separation of the three colonies. The obtained discriminant functions were applied to the other bees checking to which colony they were assigned. We found that the assigned colony could explain almost completely the behaviour observed in the colony performing acceptance errors (28 out of 30 observed behaviours). In the other two colonies, most landing bees were assigned to the colony where they landed and therefore the model explained acceptance errors but not rejection of NM or rejection of A. However, the analysis of the plots representing the single specimens according to their discriminant scores shows clearly that in both the colonies most of the rejected A (9 of 12 and 10 out of 11) and most of the rejected NM (2 out of 3 and 4 out of 6) lay outside from the cluster formed by the accepted NM. Therefore CH composition seems to explain most of the observed recognition interaction, although our discriminant analysis only explains behaviour of the more tolerant colony.

Session 6. Genes, genomes and social behaviour

Eusociality, inclusive fitness theory and the major evolutionary transitions

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The evolution of eusociality in social insects is a prime example of a major evolutionary transition. Such transitions are those events in life's history in which previously independent units teamed together to form new biological individuals (Maynard Smith and Szathmáry 1995). Other examples are the evolution of the eukaryotic cell via endosymbiosis and the evolution of multicellularity from the grouping of unicellular organisms. In this presentation, I first defend inclusive fitness theory against recent critiques (e.g. Wilson and Hölldobler 2005; Nowak et al. 2010) that have queried the cogency of the theory and its contribution to our understanding of social evolution, especially in the case of the evolution of eusociality in insects (Abbott et al. 2011; Bourke 2011a). I then argue that inclusive fitness theory is even more useful than is frequently appreciated, in that it helps elucidate many key facets of the major evolutionary transitions. As Queller (1997) showed, much can be deduced about the major transitions by classifying them into those involving relatives (fraternal transitions) and those involving non-relatives (egalitarian transitions). On top of this, each transition can, I propose, be usefully divided into three stages -- social group formation, social group maintenance, and social group transformation (Bourke 2011b). The first of these involves the processes by which social groups originate, the second the processes by which they are kept stable, and the third the processes by which they are transformed into entities resembling individuals in their own right. I argue that inclusive fitness theory illuminates the nature of all three stages, including the little-studied step of social group transformation. Overall, inclusive fitness theory has proved an effective tool for understanding not just the evolution of eusociality but the social evolutionary processes that underlie the deep structure of all life.

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Divergent experimental selection for philopatry and dispersal affects the evolution of cooperative breeding in primitively eusocial ambrosia beetles

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An important route to eusociality is characterized by delayed dispersal of offspring and cooperative care of subsequent brood by philopatric subordinates. It is yet unclear, however, how the propensities to delay dispersal and to cooperate relate to each other, and in particular, whether there is correlated genetic variance for these traits. We selected for either early or late dispersal in two lines of the primitively eusocial ambrosia beetle *Xyleborinus saxesenii*. After six generations the two lines differed significantly in the timing of dispersal. Remarkably, the strain selected for late dispersal also showed more cooperative nest protection behaviour, suggesting a genetic correlation between delayed dispersal and cooperative sib raising. Furthermore, this cooperative effort resulted in higher productivity of the line selected for delayed dispersal and cooperation. This traded off against reduced feeding rates and significant fitness costs to delayed disperses, however, when these attempted to breed independently. To our knowledge this is the first experimental proof of a significant genetic correlation between philopatric and cooperative behaviour in highly social animals.

Kin-selected conflict and the evolution of lifespan and ageing in *Bombus terrestris*: queen longevity, fecundity and queen-worker conflict

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Recent extensions to the evolutionary theory of ageing have incorporated processes of social evolution likely to affect ageing. One hypothesis stemming from this is that social systems with intergenerational resource inheritance are predicted to exhibit kin-selected, parent-offspring conflict over the timing of inheritance. Preliminary evidence exists for such kin-selected conflict in the bumblebee *Bombus terrestris*. Workers have been reported to only lay eggs in their natal colony at high frequency following the death of their queen. They have also been reported to harass and even kill their queen. Predicted fitness optima, in regards to the timing of resource handover, differ between queens and workers in colonies of *B. terrestris* as the two parties are differentially related to worker offspring. We predict, therefore, that queen longevity is causally linked to the amount of direct reproduction that workers achieve. We also predict that workers may monitor queen fecundity through, for examples, cues deposited on queen laid eggs, and increase aggression towards the queen, or begin egg-laying sooner, if these cues are experimentally diminished. Finally, we predict that those workers that are capable of reproduction will be the first to harass the queen. Our results show that (1) queen longevity is causal to the number of sexual offspring achieved by workers, (2) workers might assess queen fecundity via cues present on her eggs and subsequently increase aggression towards the queen, but do not change the timing of their egg laying when such cues are manipulated and (3) on average, workers that were observed to lay eggs were more likely to aggress towards their queen.

Kin-selected conflict and the evolution of lifespan and ageing in *Bombus terrestris*: age-associated transcription

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Recent syntheses of the evolutionary theory of ageing have incorporated social components such as inclusive fitness. These models predict kin-selected parent-offspring conflict in social systems with resource inheritance, like nest handover in the bumblebee *Bombus terrestris*. A bumblebee queen's declining fecundity is predicted to create conflicts in the desired time for nest handover, then to accelerate her ageing as all parties desire nest handover. Preliminary behavioural observations in bumblebees support predictions of the hypothesis, though this project aims to provide an empirical test. My focus is on the influence of social stimuli on age-associated transcription, and whether queen ageing is accelerated around the switch point where resource handover becomes favoured. Some genetic pathways play conserved roles in ageing, yet as longevity has arisen independently multiple times there are also some pathways of ageing which differ between animals. Oxidative stress has a conserved role in ageing: the reactive oxygen species generated by mitochondrial respiration are thought to be stress-signalling molecules between the mitochondrion and cytosol. DNA methylation and other epigenetic marks change with age in many animals, and correlate with differentially ageing castes in honeybees. Other pathways linked to ageing in insects include vitellogenin, which is involved in foraging initiation and queen/worker differences; and 'foraging' kinase whose expression decreases with age in bumblebees. This project examines changes in the transcription of key genes in these processes as a function of natural and manipulated social situations, around the resource handover switch point in bumblebee colonies. Our results suggest limited transcriptional changes associated with ageing, with some changes occurring as a function of natural or manipulated social situation.

Sociogenomics of conflict and cooperation in fire ant queens

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Founding behavior is a key feature of life history in social insects and one of the best examples of behavioral plasticity, since young queens can often adopt multiple strategies of colony foundation within the same species. Newly mated queens of the fire ant *Solenopsis invicta* can found new colonies alone (haplometrosis) or in group (pleometrosis). Here, the initial cooperation among queens, useful to defend the incipient colony against enemies and to increase the size of the colony more quickly, turns into conflict as soon as the first workers are close to emergence. Only one queen will be allowed to lead the mature colony while the others will die in fights with the dominant queen or as a consequence of the repeated aggressions performed by workers. We developed a microarray platform to monitor the expression of ~50K probes: these include a set of ~16K genes annotated from the *Solenopsis invicta* genome project, and a set of ESTs obtained from transcriptomic studies. We characterized the expression patterns in haplometrotic and pleometrotic queens. Counterintuitively, we found that the social context is more important than the outcome of the conflict: pleometrotic queens have very similar gene expression compared to haplometrotic queens. Moreover, changing the social status in pleometrotic queens from winner to loser and vice-versa triggers larger changes in gene expression than the social status itself. This means that the social environment is the major force driving gene expression in pleometrotic queens and its drastic manipulation produces effects at the genomic level.

Egf receptor determines reproductive fate of worker bees

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In honeybees, usually only the queen lays eggs, whereas the thousands of workers in a colony have poorly developed and inactivated ovaries. However, workers retain some reproductive potential; upon queen loss most of them will activate their ovaries to some degree. Until now it remained enigmatic how the sterility of worker bees in the presence (and fertility in the absence) of the queen was regulated. We identified the Epidermal Growth Factor Receptor (EGFR) as a key element in fertility (or enforced sterility) in honeybees. Upon down-regulation by RNAi, adult workers remain sterile, whereas adult workers in the control group became fertile. Since in the wild type the abundance of EGFR is the same in fertile and sterile worker bees, we suggest that the concentration of the EGFR-ligand is controlling the fertility in honeybee workers. Interestingly, EGFR is also involved in regulating the larval development towards a queen. This is the first identification of a molecular switch that controls fertility in the Hymenoptera, including ants, wasps and bees. The finding that the same pathway is active in controlling both larval development and adult ovary activation supports the Reproductive Ground Plan Hypothesis (RGPH) that states that gene regulatory networks of solitary ancestors have been coopted in the social insects.

Genomic imprinting in the honey bee methylome

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The Kin Theory of Genomic Imprinting (KTGI) predicts that imprinting will arise when the reproductive interests of parents differ [1]. In a honey bee colony a queen maximizes her reproductive success if her worker daughters are sterile, whereas a male can benefit if his daughters are fertile. One candidate mechanism by which queens may enforce sterility in their worker offspring is to epigenetically silence genes that are required for fertility using DNA methylation [2]. We will report that there is differential methylation of the genome in honey bee eggs and sperm. 318 genes show significantly different CpG methylation patterns, with the vast majority being more methylated in egg. Genes that are differentially methylated between eggs and sperm are significantly more likely to be differentially expressed between reproductively active workers and those that are sterile. These extensive methylome differences represent the first characterization of genomic imprinting in any insect and provide support for the KTGI by showing that the methylome of reproductive males and females differs strongly for a number of genes. Parentally-directed epigenetic modification of genes related to reproduction may therefore be a key mechanism by which eusociality evolves and is maintained.

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Ploidy vs sex locus: which one determines gene expression in diploid males in fire ants?

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Sex in Hymenoptera is usually determined by a single locus: unfertilized eggs always become haploid males while fertilized eggs can become diploid males (if homozygous at the sex determining locus) or diploid females (if heterozygous at the sex determining locus). In the invasive fire ant *Solenopsis invicta*, diversity at the sex locus was reduced during its introduction by humans so that diploid males are particularly common. Diploid males are sterile and larger but otherwise morphologically and behaviorally identical to haploid males. Diploid males thus provide a unique opportunity to study the role of ploidy level and heterozygosity at the sex determining locus on gene expression. We compared gene expression between diploid and haploid males and virgin queens at three developmental stages (pupae; 1d and 11d after eclosion) with cDNA microarrays. We find that diploid male pupae have almost identical gene expression profile to virgin queen pupae but have become significantly different at 1d adults. By contrast, at 11d, there is no difference between diploid and haploid males. Hence, ploidy level greatly affects gene expression profile at the beginning of development while the sex-determining locus ultimately abrogates the effects of ploidy during development.

Insights from comparative genomics into the genome and phenotypic evolution of Hymenoptera and Formicidae lineages.

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Despite longstanding interest in the genetic basis of important biological traits there has been paucity in identifying their molecular underpinnings within or across species of social insects (1). The increasing availability of genomics information for social insects can help in linking genes with phenotypes (2). In the work presented here we systematically compare genome sequences with two aims: 1) to reveal genomic innovations that are shared across social insect species and therefore shed light onto the evolution of eusocial systems, and 2) to discover innovations that are unique to specific lineages of eusociality. We focused on the evolution of protein-coding genes because they are one of the best understood genome features. We studied the genomes of the 33 fully sequenced Arthropod species, including 10 Hymenoptera and 9 social insects. We used maximum likelihood methods (3,4) to study the evolution of over 20000 gene families in the lineages leading to both Hymenoptera, broadly, and Formicidae, more narrowly. Although highly interesting, these analyses rely on fully sequenced genomes. Therefore, we complemented our analyses by using the PhyloStratiphy methodology (5), which describes the dynamics of gene birth, to compare genomes of both groups with those of Arthropods. We discuss the biology of our findings from three perspectives: a) the individual gene families which likely expanded or contracted in the lineages leading to Hymenoptera and Formicidae; b) using systems biology tools we highlight gene pathways of interest for developing an understanding of the cellular and physiological contexts of these changes; and c) we will point to evolutionary periods during which social insects' genomes likely experienced major genomic innovations, which could serve as raw material for the evolution of complex traits. Comparative genomics offers powerful tools to explore and synthesise the incoming wave of information from social insects' genomes.

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Comparative genomics of fungus-growing ants

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The fungus-growing attine ants represent one of the most spectacular examples of mutualistic partnership in nature. They provide their fungal symbionts with nutrients in the form of organic plant material and with a stable and protected growth environment. In return their farming efforts give them a predictable food source in the form of fungal hyphae. The attine ants are monophyletic and evolved about 50 million years ago. Their later history is characterized by two major evolutionary transitions, the first happening ca 20 MYA with the origin of specialized fungiculture focusing on a single co-evolving clade of symbionts, which allowed colony size to increase by at least an order of magnitude. The second happened 10 MYA when a single lineage of the higher attine ants adopted active herbivory in association with a single species of fungal symbiont with special abilities to handle fresh leaves. This transition was accompanied by a further substantial increase in colony size, the development of polymorphic worker castes, and multiple-mating by queens.In order to reveal the genomic changes associated with these two major evolutionary transitions, we have completed ca. 100x coverage genome sequences and overall transcriptomes for six species, representing the major branches of the higher attine ants (*Atta colombica*, *Acromyrmex echinatior*, *Trachymyrmex septentrionalis*, *T. cornetzi*, and *T. zeteki* – a branch that includes *Sericomyrmex*) plus *Cyphomyrmex costatus* as lower attine outgroup. We will present the first results of our comparative analyses of these genomes, including genome wide differences in gene family expansions/contractions and positively selected single genes, as well as more targeted comparisons of single genes known to be involved in specific biological processes that differ among the attine lineages.

Attine ants: the animal freight trucks of their fungal symbiont. New molecular insight into a complex ant-fungus mutualism

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Research on the mutualism between fungus-growing ants and their symbionts normally views the ants as farmers and the fungi as crops (Weber, 1966). However, there is growing evidence suggesting that the fungus is actually using the ants as a live transportation device for providing substrate and transporting fungal enzymes to the new leaf material that the ants add to their fungus gardens. The ants manure gardens with their own fecal fluid, which is packed with fungal enzymes for the biodegradation of the leaf pulp that the ants deliver (Martin, et al. 1975). Recent studies have shown that fungal pectinases in the fecal fluid serve complex adaptive functions in the symbiosis. The genes coding for these enzymes are often overexpressed in the gongylidia, the swollen hyphal tip structures on which the ants feed, which is consistent with a scenario of advanced co-adaptation (Schiøtt et al., 2010). Here we show that also endopeptidase and endocellulase activities in the fecal fluid of the leaf-cutting ant *Acromyrmex echinatior* have a fungal origin. We characterized the genes encoding these enzymes using a partially sequenced genome of the fungal symbiont *Leucocoprinus gongylophorus* as reference database, and quantitative RT-PCR to show that also these genes are overexpressed in the gongylidia. Finally, the enzyme mixture in the fecal droplets appears to have a sequential order of activity with a single endocellulase opening the cellulose matrix in the leaf-cell-walls, followed by the rapid breakdown of cell-wall pectins by multiple fungal pectinases after which the fungal endopeptidases digest the cell-content proteins. Our results corroborate that the ant-fungus symbiosis is highly integrated and that complementary roles controlled by the fungal symbiont deserve more explicit emphasis.

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DNA methylation and caste differentiation in ants

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Ant societies have individuals belonging to different castes that express specific behaviors or have different adult mophologies. Ants have a full set of DNA methyltransferases and their genomes contain methylcytosine, so it is reasonable to infer that epigenetic mechanisms play a role in caste determination. Genome-wide methylomes of different castes and developmental stages of *Camponotus floridanus* and *Harpegnathos saltator* show that methylcytosines are found both in symmetric CG dinucleotides (CpG) and non-CpG contexts and are strongly enriched at exons of active genes. The same data also reveal that changes in exonic DNA methylation correlate with alternative splicing events such as exon skipping and alternative splice site selection. Several genes exhibit caste-specific and developmental changes in DNA methylation that are conserved between the two species, including genes involved in reproduction, telomere maintenance, and noncoding RNA metabolism. Several loci also appeared to be methylated and expressed monoallelically, sometimes in a caste-specific manner. This suggests that parental or caste-specific genomic imprinting may play a role, consistent with theory predicting that genomic imprinting should be common in ants and other hymenopteran social insects. The fungus growing attine ants are a promising clade to further address questions about methylation and imprinting, because reference genomes of all phylogenetic branches of the higher attine tree are now becoming available, spanning one of the best studied transitions from single-to multiple queen mating in any social insect.

Genetic compatibility affects division of labor in ants

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Division of labor is central to the organization of insect societies and stems from between-worker differences in behavior and task performance. Over the last decades, within-colony behavioral comparisons of subfamilies of workers (patrilines or matrilines) revealed genetic effects on division of labor in many species of social insects. Although this has been taken as evidence for additive genetic effects on division of labor, it remains unknown whether such effects really exist. To determine the relative roles of additive and non-additive genetic effects (e.g., epistasis) on worker behavior, we performed controlled crosses in the laboratory using the Argentine ant *Linepithema humile*. We measured the maternal and paternal effects, as well as interactions between these effects on the efficiency of workers to feed larvae and collect pupae, their foraging propensity and their average distance to the brood. Our results reveal that all these measures of worker behavior were affected by only one of the parental genetic backgrounds and/or by the interactions between the maternal and paternal genetic backgrounds. The finding of effects of only one of the two parents and widespread interactions between the parental genomes is most consistent with non-additive genetic effects, such as genetic compatibility effects, on behavior. This is in strong contrast with the current view and has important consequences for our understanding of division of labor in insect societies.

Raising royalty: is emergency queen selection related to larval growth in *Apis mellifera*?

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Division of labour is a fundamental property of eusociality and so the mechanism underlying caste determination is one of the most intriguing questions in social insect biology. Although traditionally thought to be determined solely by the larval environment, a genetic component to caste determination has been shown in an increasing number of species. Unsurprisingly, there is no consistent pattern; the interaction between environmental and genetic effects is not constant across study organisms. In the polyandrous honeybee, *Apis mellifera*, caste is strongly influenced by the environment, and queens are fed an exclusive diet which is unavailable to developing workers. During emergency queen rearing, however, workers select larvae to be reared as replacement queens. A number of studies have shown that within a colony, some patrilines (paternal lineages) are disproportionately represented as emergency queens, but the reasons underlying such observations have been the subject of much debate. Here, we use measurements from larvae reared *in vitro* to investigate whether patrilines differ significantly in their growth rate, and consider whether the selection of larvae by workers for emergency queen rearing is related to patriline growth rate. As in previous studies, we found there was significant variation between patrilines in their propensity to be reared as queens. However, we did not find any significant variation between patrilines in their growth rates. Our results support the suggestion that caste-determination in *Apis mellifera* is a result of a complex interaction between nurse bees and brood, rather than simply being due to intrinsic variation in growth.

Effects of natural mating and carbon dioxide narcosis on biogenic amine receptor gene expression in the ovaries and brain of queen honey bees, *Apis mellifera*

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A queen honey bee mates at around 6 days of age, storing the sperm in her spermatheca for life. Mating is associated with profound changes in the behaviour and physiology of the queen but the mechanisms underlying these changes are poorly understood. What is known is that the presence of semen in the oviducts and spermatheca is insufficient to initiate laying, and that copulation is necessary for ovary activation. In this study we use real-time quantitative PCR to investigate the expression of biogenic amine receptor genes in the brain and ovarian tissue of queens in relation to their mating status. We show that dopamine, octopamine and serotonin receptor genes are expressed in the ovaries of queens, and that mating, carbon dioxide narcosis, and the presence of semen in the spermatheca differentially affect their expressions. We suggest that these changes may be central to the hormonal cascades that are necessary to initiate oogenesis.

Queen pheromone blocks responsiveness to alarm pheromone in bees.

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When the hive is threatened, honey bee (*Apis mellifera*) guards sound the alarm by producing alarm pheromone [1] that elicits a collective defense through recruitment of many workers. Recently alarm pheromone was shown to modulate more complex behaviors and specifically to inhibit appetitive learning [2] in bees of foraging age. However, only older bees take part in the defense of the hive; young bees, although they are able to perceive the pheromone, are not recruited by this signal [3]. This age polyethism is well known: bees undergo profound behavioral and physiological changes as they age [4-6]. For instance only young bees exposed to the queen mandibular pheromone (QMP) have impaired aversive learning abilities, older animals are not affected by this treatment [7]. We therefore tested if the effect of alarm pheromone on learning was also age-dependent. We show here that alarm pheromone fails to impair such learning in young bees. As young bees are more likely to come into contact with the queen and are more exposed to her pheromones than older bees [8], we examined whether QMP inhibits responses of young bees to alarm pheromone. Blocking the responsiveness of young bees to alarm pheromone would be a way for the queen to ensure constant care even when bees are recruited to defend the colony. We will present age-related changes in level of expression of target genes, both in bees exposed to QMP and raised without it, in parallel to the behavioral effect of alarm pheromone on appetitive learning.

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Aversive conditioning with heat punishment in the honeybee (*Apis mellifera*): behavior and genetics

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In nature, animals associate initially neutral sensory stimuli (color, odor, etc.) with positive (food) or negative events (danger), based on appetitive and aversive learning respectively. The honeybee (*Apis mellifera*) is an invertebrate model commonly used in the Laboratory for studying olfactory learning and memory. Until recently, research almost exclusively focused on olfactory appetitive learning, using the protocol for conditioning the proboscis extension response (PER), in which bees learn to associate an odor (conditioned stimulus - CS) with a sucrose reward (unconditioned stimulus - US). A few years ago, a new aversive learning protocol was developed. In the conditioning of the sting extension response (SER), bees learn to associate an odor (CS) with an electric shock (US). The electric shock is however not a natural aversive stimulus for bees and it is therefore difficult to identify the peripheral receptors involved. We thus aimed to provide a more natural and controllable US for SER conditioning. Here, we tested the use of a heat probe applied locally to the bees' body parts. We show that stimulation of most regions of the bee body with heat elicits the SER, in a temperature-dependent manner. Moreover, heat applied to the antennae, mouthparts or legs can be used as US in SER conditioning procedures.
Honeybees are also a good experimental model for studying the impact of genetic variations on learning and memory. A queen bee usually mates with 15-20 drones, so that her worker offsprings belong to as many different patrilines. Using microsatellite analysis, we analysed heat sensitivity and aversive learning performances in worker patrilines issued from a naturally inseminated queen. Future work will compare aversive and appetitive conditioning in the same patrilines, to understand whether both types of learning depend on the same or on a different genetic basis.

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Genes behind chemical communication? – Rapid evolution and positive selection in the CSP gene family of ants

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Ants and other social insects have sophisticated chemosensory abilities because they use chemical communication in social context and much of their interaction relies on chemicals. We studied if this complexity is reflected in the evolution of chemosensory protein (CSP) gene family either as an increase of gene number or positive selection at molecular level. CSP's are similar to odorant binding proteins (OBP) in that they are small, soluble binding proteins and secreted into the lymph of insect chemosensory sensilla, but they are also expressed elsewhere in the body. One of the CSP's has been shown to selectively bind the cuticular hydrocarbons used in nest-mate recognition in *C. japonicus* (Ozaki et al. 2005). Another CSP is the major protein expressed in *S. invicta* antennae and could be the protein used in binding nestmate recognition substances, though not hydrocarbons (González et al. 2009). We searched the seven sequenced ant genomes using previously identified insect CSP sequences and found that the CSP gene family has expanded in ants (11 to 21 functional genes) compared to honey bee (six genes) and *Drosophila* (four genes). At the gene family level, the birth-and-death rate (λ) of the CSP gene family is higher in social than in other insects. Furthermore, ant CSP genes can be divided into two groups; conserved orthologous genes (global dN/dS=0.28) and rapidly evolving lineage specific genes (dN/dS=0.57). Thirteen sites have been under positive selection in these rapidly evolving genes. Especially interesting is an expansion of six genes in *S. invicta*, including the major antennal protein, which shows an extremely rapid rate of molecular evolution (dN/dS=1.2) and 24 sites that have been under positive selection. The ant-specific expansions, the higher birth-and-death rate and rapid evolution connected to positive selection suggest adaptive evolution of CSP genes in ants.

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The role of microRNAs in caste determination and differentiation in the eusocial insect *Bombus terrestris*

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Eusocial insects are species that exhibit a reproductive division of labor that results in individuals that develop into separate castes. In the Buff-tailed bumblebee, *Bombus terrestris*, this means that the females are capable of developing into either reproductive 'gynes' or non-reproductive 'workers', and the developmental trajectory towards either caste is dependent on the environmental interactions that the individual is subjected to as a developing larva. Previous research on *Bombus* and on other eusocial insects has started to identify the molecular basis of caste development in order to understand how completely different phenotypes can emerge from a single genotype (Pereboom et al. 2005, Barchuk et al. 2007). These studies have shown that it is often the timing and regulation of multiple interacting genes at different stages of development that is critically important in deciding an individual's caste fate. MicroRNAs (miRNAs) are a relatively recently discovered class of small (~22 nucleotide) regulatory RNAs that control the translation of mRNA into proteins and they therefore play an extremely important role in development and gene regulation in higher eukaryotes (Behura 2007). This means that they may be important regulators of caste differentiation in eusocial insects. In this study we aimed to identify whether miRNAs do have an important role in caste differentiation, and which miRNAs might interact with genes that are differentially expressed between castes. To do this we collected RNA from 'worker' and 'queen' larvae at different stages of development and used deep sequencing to identify all of the potential miRNAs which are likely to play a role in deciding an individual's caste. Here we discuss the initial sequencing findings and discuss further directions on the molecular basis of caste differentiation in *Bombus*. This may provide future insights into the molecular basis of social behavior in Bumblebees and other eusocial insects.

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The genetic basis of reproductive division of labor in *Temnothorax* ants: First results of a gene expression study

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Reproductive division of labor is a key feature of social Hymenoptera. Although queens and workers share the same set of genes, they strongly differ in morphology, physiology, behavior and longevity. The queen typically reproduces, while sterile workers tend the brood, guard the nest and search for food. Colonies of the facultative polygynous ant *Temnothorax longispinosus* contain only few monomorphic workers, which can be nevertheless assigned to distinct behavioral castes. Following queen removal, young brood tending workers start to develop ovaries and engage in dominance interactions. The comparison of queens – reproductive and sterile workers thus allows to compare a continuous gradient of reproductive status specific gene expression patterns. The results are compared to a similar study, previously conducted in the honey bee.

The expression of aging: gene-expression differences underlying the disparity in lifespan between queen and worker ants.

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A defining characteristic of advanced eusocial societies is the morphological differentiation that distinguishes queens from workers. The specialisation of the morphology to suit specific tasks, such as risky foraging or egg-laying, is accompanied by an equally specialised physiology highly adapted to these tasks. Among the important elements of this physiology is the process of aging, and caste differentiation in aging profiles is demonstrated by the huge differences in lifespan exist between queens and workers. Workers, who carry out risky activities such as foraging and who, at least in monogynous colonies, are more dispensable, live much shorter lives than queens, even in laboratory conditions. In the ant *Lasius niger*, for example, the queen may live as much as 15 times longer than workers. The genetic basis of this differences is, however, still poorly understood. In order to investigate the gene expression patterns that underlie these differences in aging, we perform RNA sequencing in age-controlled cohorts of *L. niger* queens and workers, focusing in particular on differences in genes involved in somatic maintenance, such as DNA repair mechanisms and genes involved in maintaining protein integrity. Furthermore, the ubiquitousness of the data will allow us to globally examine the differences in gene expression profiles between queens and workers and how they relate to the task specialisations that form the basis of such advanced societies.

miRNAs ame-miR-9c, ame-miR-3747b and ame-miR-306 are potential regulators of *EcR*, *usp* and *ftz-f1* expression in *Apis mellifera* queen ovaries

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Honeybees (*Apis mellifera*), as eusocial Hymenoptera, are characterized by overlapping generations, cooperative brood-care and reproductive division of labor. Their colonies consist of one queen, thousands of workers and hundreds of drones; and the queen is the only female capable of mating and laying diploid eggs. Mating is relevant to ovary activation and resulting oogenesis. Concerning the hormonal aspects, the 20-hydroxyecdysone (20E) also participates in reproductive processes in various insect species. This hormone acts through the ligation to its receptor, a dimer of EcR and USP (ultraspiracle), which recognizes specific DNA sequences and regulates the transcription of many genes, including *ftz-f1*, an important gene to *vg* (main egg protein) transactivation in *Aedes aegypti*. To investigate the role of these genes in honeybee reproduction, we analyzed the expression of *EcR*, *usp* and *ftz-f1* and their putative regulator miRNAs in ovaries of virgin and mated queens by qPCR. Our results show that the levels of transcripts of *EcR*, *usp* and *ftz-f1* are higher in virgin queens when compared to reproductive ones. Using the software RNAhybrid, we searched their 3'UTR regions for potential miRNA binding sites. For *EcR*, *usp* and *ftz-f1*, we identified 18, 13 and 17 candidates, respectively. For expression analysis, we selected 9 miRNAs (ame-miR-9c, ame-miR-1, ame-miR-276, ame-bantam, ame-miR-3747b, ame-let-7, ame-miR-305, ame-miR-275 and ame-miR-306) of our interest. The miRNAs, ame-miR-9c, ame-miR-3747b and ame-miR-306, were higher expressed in mated queens when compared to virgin ones. As they have antagonistic expression patterns compared to their putative targets, we presume that they are strong candidates to down-regulate *EcR*, *usp* and *ftz-f1*, respectively. All the other miRNAs showed higher expression in virgin queens. We consider that this study provided new important information regarding the molecular pathways involved in ovary activation and this is the first report concerning the regulation of *EcR*, *usp* and *ftz-f1* by miRNAs related to this issue.
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Variation in caste specific Vitellogenin expression within and among colonies of Formica ants

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The availability of gene sequence and gene expression data increasingly allows investigating how natural selection works at its most fundamental level. Gene expression differences between queens and workers are crucial for social insect evolution, but when relatedness is low the very same gene expression differences make the gene expressed in workers vulnerable to mutation accumulation. This is because the strength of selection on genes expressed in workers only decreases when relatedness between queens and workers decreases. However, evolutionary stability of caste biases in gene expression is still unknown. We aim to investigate whether genes vary in stability of their expression pattern across closely related species, and whether this variation is linked to kin structure of the species.
Little is known about the variation in caste biased gene expression among closely related species, and among colonies within species. Furthermore, in a natural environment, gene expression differences between individuals within the same colonies may arise due to external, uncontrolled factors, which are often not taken into consideration when pooling individuals for gene expression studies. In this study we investigate how caste biased expression patterns of Vitellogenin vary among individuals, colonies and species in *Formica* ants where relatedness within nest varies between closely related species. Caste biased expression of Vitellogenin is well known to affect several aspects of social insect life history and behavior. This study will provide insight into variation in caste specific gene expression across species and across colonies of social insects and will contribute to improve further gene expression studies.

Signatures of divergence and selection in *Cardiocondyla obscurior*

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The tramp ant species *Cardiocondyla obscurior* is distributed across the tropics and subtropics. For introduced species, colonization events are associated with genetic bottlenecks drastically reducing allele diversity in the population; a phenomenon referred to as founder effect. Studies comparing two introduced populations of *C. obscurior* from Brazil and Japan revealed significant phenotypic differences (e.g. CHC profiles), indicating an early stage of divergence. Furthermore, crossing experiments between the two populations showed fitness defects in outbred queens. This susceptibility to outbreeding depression might be related to the evolutionary history of *C. obscurior*, in which inbreeding in the maternal nest is favoured. The founder effect of low genetic diversity together with the susceptibility for outbreeding depression in *C. obscurior* provides a framework for studying the evolution of genetic incompatibility and of the very early steps of speciation processes. By comparing the sequenced genomes from the two different populations we aimed to identify genetic differences and signatures of selection potentially accounting for the emerging incompatibility.

Intra-organismal competition in chimeric colonies of the clonal ant *Cerapachys biroi*

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Chimeric organisms are expected to host intra-organismal conflicts. Studies on social micro-organisms show that competition over reproduction arises in chimeric aggregations of different clonal lines. Owing to its unique biology, we were able to test these predictions in the ant *Cerapachys biroi* and investigate for the first time this type of conflict in a metazoan model. In this species, all the individuals in a colony lay eggs through thelytokous parthenogenesis. Colonies are therefore typically monoclonal, and several clonal lines occur in the wild. Two categories of individuals exist in the colonies: Lowly Reproductive Workers (LRWs), and Highly Reproductive Workers (HRWs). These two categories or castes show differences in the number of ovarioles (2 in LRWs and 4 to 6 in HRWs), different reproductive potentials (number of eggs laid) and distinctive features in external morphology. The production of HRWs is regulated through a feedback system and depends on the proportion of fertile individuals already present in the colonies. Experimental colonies composed of individuals from two different clonal lines were reared until they produced six cohorts of brood. Individuals of various cohorts were then genotyped and categorised into HRWs and LRWs in order to assess whether an inter-clonal reproductive competition occurred. Moreover, in order to have a sharper picture of the reproductive behaviour of each clone in competitive or non-competitive contexts, we conducted a series of cross-fostering of freshly hatched larvae between the two clonal lines. Our results showed that in a competitive context one clonal line always produces more HRWs. We suggest that this differential competitive ability depends on 1) the different levels of inhibition, by adults of different clones, of the HRW developmental pathway, and 2) the differential thresholds of larvae of different clones for developing in HRWs. These specificities of the different clonal lines may represent different reproductive strategies.

A Y-like social chromosome causes alternative colony organization in fire ants

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Intraspecific variability in social organization is common, yet the underlying causes are rarely known. In the fire ant *Solenopsis invicta*, the existence of two divergent forms of social organisation is under the control of a single Mendelian genomic element marked by two variants of an odorant binding protein gene. Here we characterize the genomic region responsible for this important social polymorphism and show that it is part of a pair of heteromorphic chromosomes having many of the key properties of sex chromosomes. The two variants, hereafter referred to as the Social B and Social b (SB and Sb) chromosomes, are characterized by a large region of ca. 13 Mb (55% of the chromosome) where recombination is completely suppressed between SB and Sb. Recombination appears to occur normally between the SB chromosomes but is impossible between Sb chromosomes because Sb/Sb individuals are non-viable. Genomic comparisons revealed limited differentiation between SB and Sb, with the vast majority of the 616 genes identified in the non-recombining region present in the two variants. The lack of recombination in this large region is likely due to Sb-specific repetitive elements and inversions. Importantly, the non-recombining region comprises most of the genes with demonstrated expression differences between individuals of the two social forms. These findings highlight how genomic rearrangements can maintain divergent adaptive social phenotypes involving many genes acting in concert by locally limiting recombination.

Sex specific gene expression and haplodiploidy purging

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In populations with small effective sizes (N_e), the loss of allelic variation increases the risk of inbreeding depression by expression of harmful or even lethal recessive alleles in diploid individuals. However in hymenoperans only females are diploid whereas males are produced from unfertilized haploid eggs. Thus harmful recessive alleles are routinely exposed to natural selection and likely purged from populations when expressed in hemizygous males. Due to this haplodiploidy purging it has been hypothesized that hymenopterans are less prone to inbreeding depression than diploid species.
In this project we aim to use RNA-seq to identify genes that exhibit preferential male or female (queens and workers) expression in the ant *Formica exsecta*. We then compare nucleotide variation in the coding region of a subset of these genes among North-European populations of the species. Given haplodiploidy purging, we predict that genes which are commonly expressed in males have on average fewer non-synonymous nucleotide changes that affect conversely the protein structure than genes which are never expressed in males.

Session 7. Functional morphology and systematics

How *Pyramica sauteri* ants use a novel exocrine gland for prey capture

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Ants of the tribe Dacetini display a peculiar behaviour during prey capture. They often feed on Collembola, that are often approached in a stealthy way. This involves a very slow approach of the ant towards the prey, which seems to be attracted by a transparent secretion on the anterior tip of the ant's head (Masuko, 1984; Dejean, 1985, 1986). We studied this behaviour in *Pyramica sauteri*, in which this clypeal region appears as a platform-like shield. Light, scanning and transmission electron microscopy of this anterior head region revealed the presence of a novel clypeal gland, that is made up by class-3 glandular cells that open at the upper clypeal surface through small pores. Behavioural observations confirmed that collembola were attracted to this anterior head area, where the gland pores open. We observed prey licking the clypeus of the crouching, motionless worker. As this brings the collembola within the reach of the ant's mandibles, it will be seized and carried to the ant nest. Using a SPME-fiber fixed to a micro-injector, we could perform precise small area sampling followed by chemical analysis. This showed a similar hydrocarbons pattern of the various head regions that were analysed, but also reveals that the upper clypeal region of foraging ants contains two additional compounds, that may play a role as prey attractants.

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Using Multiple Factors to Classify Ant Species in the bicolor group (*Cataglyphis* sp. Formicidae: Formicinae)

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The bicolor group of the genus *Cataglyphis* has been studied extensively due of its unique biology. However, it is difficult to taxonomically classify the species within this group based on morphological characteristics alone. Previous work has examined species differentiation based on DNA, habitats, or chemical analysis of glandular secretions (mainly PPG) and cuticular hydrocarbons (CHC). The wide variety of species of the bicolor group that are present in Israel and their distribution within different habitats, allowed us to sample extensively and combine all of the above methodology in order to characterize the main factors in species classification, as well as construct a phylogeny. While most species within the bicolor group seem to be parapatric in Israel, we believe that some species overlap exists, the study of which can give us an insight into the evolution of species differentiation within the bicolor group.

Neuroanatomical study of the antennal lobe of the invasive Asian hornet *Vespa velutina*

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Vespa velutina is an Asian hornet which was accidentally introduced in France before 2004. This invasive species preys on numerous insect species, but predominantly from honeybee colonies, eventually leading to their demise. The hornet is well adapted to our west European climate and extends its territory each year. Its establishment in European countries (France 2004, Spain 2010, Belgium and Portugal 2011) could have a negative impact on the environment (predation of /competition with other insect species) as well as on human activities (esp. beekeeping). Olfaction plays a key role in the biology and behavior of social insects. Therefore, control methods interfering with hornet's olfactory behaviour could be promising for limiting its invasion without impairing biodiversity. Mass trapping with specific odorant baits could be one of these solutions. This however requires acquiring a good knowledge of this hornet's olfactory system. In insects, odorant molecules are detected by olfactory sensory neurons (OSN) on the antennae, which project to a primary olfactory structure, the antennal lobe. It is made of morphologic and functional units called 'glomeruli' which each represent input from a given type of OSN. In males but also workers of some insect species, enlarged glomeruli called 'macroglomeruli' are involved in the detection and processing of pheromonal signals. Finding macroglomeruli in hornets may indicate the use by males or workers of specific and potentially attractive chemical signals. Here as a first step, we used anterograde antennal staining, confocal microscopy and 3D reconstruction to describe the antennal lobe of the different sexes and castes of a hornet colony. This neuroanatomical study is the first step towards understanding intraspecific communication in *Vespa velutina.* Using an integrative approach combining neuroanatomy, functional imaging, chemical ecology, behaviour and field studies we aim to find highly attractive compounds for specific trapping of *Vespa velutina* hornets.

Functional morphology of the mandibular gland of queens of the ant *Monomorium pharaonis* (L.)

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In this study, the functional morphology of the mandibular glands of pharaoh ant queens is investigated through histological and behavioural observations. The mandibular glands of queens of different age stages and mating status were examined at the light microscopical as well as the ultrastructural level. The results clearly show a high activity of the gland at the time of hatching, followed by a rapid degeneration independent of the queens' mating status. We therefore hypothesize the glandular secretion serves to stimulate workers to remove the queens' pupal skin. Experiments to compare hatching ability of isolated pupae and pupae assisted by workers confirmed the necessity of worker assistance, which is even more crucial to allow proper wing formation in queens and males. Together with the histological data, this suggests that the mandibular gland may indeed play a role in the social facilitation of hatching, although bioassays are required to give decisive answers about the mandibular gland's function.

Evolution of the frontal gland in termite imagoes

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Frontal gland is a unique defensive organ of three most advanced termite families Rhinotermitidae, Serritermitidae and Termitidae. It occurs in alate imagoes, soldiers (and presoldiers) and rarely in workers (Šobotník et al. 2010). It is an unpaired secretory organ opening at the frons. While there is a good knowledge about the frontal gland in soldiers, other castes received only negligible attention. We have studied the development of the frontal gland in alate imagoes of following taxa: Rhinotermitidae (Coptotermitinae, Heterotermitinae, Prorhinotermitinae, Psammotermitinae, Termitogetoninae, Rhinotermitinae), Serritermitidae (*Glossotermes*) and Termitidae (Sphaerotermitinae, Macrotermitinae, Foraminitermitinae, Apicotermitinae, Termitinae, Syntermitinae and Nasutitermitinae). In *Glossotermes* and Rhinotermitidae, the frontal gland is well-developed; it is always saccular in shape, except for *Psammotermes*, in which it occurs as an epidermal thickening only. The frontal gland can be quite large, as in all Rhinotermitinae, where it reaches deep into the abdomen. The frontal gland of Termitidae imagoes is always confined to the head only. Nearly all representatives possess a functional frontal gland, which is either saccular as in many Macrotermitinae and Foraminitermitinae or reduced into epidermal thickening as in other termitid subfamilies. The frontal gland was lost in *Protermes* and *Microtermes* (both Macrotermitinae) and in *Coatitermes clevelandi* (Nasutitermitinae). The plausible evolutionary scenario is sketched and discussed in the context of modern phylogenetic hypotheses.

Šobotník J., Jirošová A., Hanus R. 2010. J. Insect Physiol. 56: 1012-1021

Session 8. Basic research on honeybees and applicative outputs

Persistence to unrewarding feeding locations by forager honey bees (*Apis mellifera*): the effects of experience, resource profitability, and season.

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Animals will often revisit previously-rewarding locations and give up on locations that are unrewarding (Gende & Sigler 2006; Van Gils et al 2003). In honey bees each forager has to decide whether to continue foraging at its patch, including whether or not to recruit additional foragers (Frisch 1967; Seeley 1995), or, if the patch is deteriorating, whether to abandon it (Townsend-Mehler & Dyer 2012; Townsend-Mehler et al 2011; Seeley 1995). Many factors determine the quality of a nectar patch, but an important currency is energy (Frisch 1967; Seeley 1995), which depends mainly on sugar concentration, the distance of the patch from the colony, and the time taken for a forager to collect a load. The aim in this study was to investigate how the previous experience of honey bee foragers at a feeding location affects their persistence at that location once food is no longer available. We hypothesized that persistence would be greater to locations that were more rewarding (closer to the hive, higher concentration of sucrose solution), for which a bee had greater prior experience (0.5h versus 2h training access), and at times of the year of lower nectar availability in the environment. We studied individually-marked worker bees from 4 colonies trained to sucrose-solution feeders. Our results support the predictions. Persistence, measured both in duration (days) and number of visits, was greater to locations that previously offered sucrose solution of higher concentration (2 v. 1molar) or were closer to the hive (20m v 400m). Persistence was also greater in bees which had longer access at the feeder before the syrup was terminated (2h v. 0.5h), and in the season of the lowest nectar availability in the environment.

Seasonal variation in honey bee foraging distance demonstrates critical gaps in food availability

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Although the proportion of our diet depending on animal pollination has increased 300% in the past 50 years (Aizen and Harder, 2009), pollinators, especially honey bees (Carreck and Neumann, 2010), continue to decline in North America and Europe (Potts et al., ; Biesmeijer et al., 2006; Kluser et al., 2010). Honey bees face particular challenges from pests (Amdam et al., 2004), pathogens (Cox-Foster et al., 2007; Higes et al., 2009), and pesticides (Henry et al.). However, independent of these issues is another major factor affecting wildlife: changes in the landscape have resulted in less available food. In response, both government and private companies have instigated initiatives to increase available forage; are these movements directing aid appropriately? Here we use a unique feature of the honey bee, the waggle dance, to make a two year-long, landscape-wide survey of foraging conditions. Our results demonstrate that summer is surprisingly the most challenging time for bees to find food. By decoding over 4000 waggle dances to natural forage sites, we show that foraging distance, which directly reflects flowering availability, significantly varies with month: honey bees forage at an average distance of 4km in July and August versus 2km in September and approximately 1 km in March. Given that flight is costly and that honey bees make economic foraging decisions that are communicated through their dance language, we demonstrate here that bees are finding it 4-10 times harder to find food in the summer compared to the spring or even autumn. Our results suggest that temperate regions like Europe and North America with strong spring flowering, some autumn flowering, and a landscape largely dominated by monocultures are presenting seasonal, summer challenges to bees.

Genotype-enviroment interactions in the autochthonous Italian peninsula honey bee subspecies (*A. m. ligustica)*.

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Three *A. m. ligustica* subpopulations were compared in three Italian habitats differing in flora and climate, in order to investigate genotype – environment interactions (GxE). The observed parameters were spring development and honey production of the colonies. The results from a total of 165 colonies showed significant GxE for the considered traits. Interestingly, for two of the considered origins, colonies produced most when kept in their region of origin. Honey production can be considered a measure of adaptation to environment, as it reflects the ability of a colony to make the most profit of the plant nectar sources present in its surroundings. The presence of GxE can be interpreted as the indication of the presence of locally adapted populations of *Apis mellifera ligustica*, despite widespread use of commercially produced honey bee queens.

Emulation of collective honeybee behaviour by a swarm of simple robots

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Young honeybees prefer a temperature of 36℃ in the hive. This can be interpreted as a mechanism to confine them to the broodnest. However, we found that in an experiment under dark conditions in an arena, single young honeybees (*Apis mellifera*) are rarely able to locate an area of their preferred temperature, whereas a sufficiently large group of bees is capable of collectively finding the right spot. To investigate this behaviour we plan to construct temperature-sensors for autonomous swarm robots and program them to emulate the behaviour of young honeybees in a temperature field. Like the honeybees in our experiments, the robots will be very limited in their capabilities. During the honeybee-experiments it was also found that the bees can be put into four different behavioural categories: Random Walkers, Goal Finders, Wall Followers and Immobile bees, whose locomotion behaviour differs by only a small number of parameters. We will exploit this individual variability by implementing a new programming paradigm: the swarm level programming paradigm. This paradigm defines the composition of a goal-tailored swarm of robots or, at a later stage, real honeybees by selecting agents with different behavioural characteristics in order to achieve a desired ultimate collective behaviour. This approach will not only allow us to create swarms of simple robots that collectively complete complex tasks but will also provide feedback to biological swarm research in that it will allow us to test various hypotheses on the fundamental individual mechanisms that determine ultimate swarm behaviour.

Hygienic behavior in honey bees: influencing factors and behavioral correlates

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Hygienic behaviour in honey bees is a form of social immunity (Wilson-Rich et al. 2009) defined as the detection and removal of diseased brood by adult workers. This behaviour naturally occurs in 10-12% of colonies (Spivak and Gilliam 1998) and is quantified using the "freeze-killed brood assay" with liquid nitrogen (Spivak and Reuter 1998). As hygiene may be very important for commercial beekeepers, we investigated the impact of external factors like the presence of food and brood across the season on the probability of hygienic behaviour. Our results demonstrate that hygienic behaviour is a generally stable trait but declines in early summer if brood is added to colonies without providing additional nutrition. Additionally, since beekeepers are keen to breed for hygienic colonies but not at the cost of having aggressive strains, we evaluated in a commercial apiary some possible behavioural correlations between hygienic behaviour and other traits, such as calmness on the comb and defensivity. Preliminary result show that there is no correlation between hygienic and stinging behaviour, confirming previous findings (Rothenbuhler 1964, Spivak and Reuter 1998), and suggests that it is possible to breed bees that are both docile and hygienic.

Interspecific within-host competition between *Ascosphaera apis* and *Aspergillus* spp. parasites in honey bee larvae.

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Parasite infections often consist of multiple parasite species or strains that compete within the host. These competitive interactions, combined with host resistance, are major drivers in the evolution of parasite strategies for survival, growth and fitness. The outcomes of these interactions are highly variable due to many factors including host immune responses, life stages, and the frequency, temporal spacing and order of each strain inoculation. Microbiological studies of honey bee hive substrates reveal high prevalences of opportunistic *Aspergillus* spp. parasites yet little is known about how these fungi interact with other obligate brood parasites such as *Ascosphaera apis* and the potential impact of multiple infections on virulence evolution. In this study *in-vitro* reared honey bee larvae were exposed to single and dual doses of *A. apis* and *Aspergillus* spp. to investigate how interspecific competitive interactions affect parasite growth, fitness and virulence. Infection parameters were quantified using sporulation and mortality rates combined with qPCR methods to measure within-host growth dynamics of each parasite. Compared to single infections, *A. flavus*/*A. phoenicis* and *A.flavus*/*A. fumigatus* treatments showed synergistic effects on larval mortality rates with *A. flavus* almost completely outcompeting coinfecting parasites in both cases. All other dual treatments showed inhibitory or slightly less than additive effects on larval mortality. *A. apis* was the only species which had an inhibitory effect on *A. flavus* growth and sporulation. These results illustrate the significance of within-host competition in the epidemiology and evolution of parasitic infections and provide insight into the complexity of inter-pathogen interactions.

The Trojan Hives: Harmful Pollinator Pathogens, Imported and Distributed in Bee Colonies

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The pollination services provided by bumblebees and honeybees are of great economic and ecological importance. Recent large-scale declines in honeybees and North American bumblebees appear to be in part driven by pathogens. Over the past three decades the increasing use and worldwide distribution of commercially reared bumblebees has increased the risk of exposure of wild bees to non-native pathogens. Here, commercial bumblebee colonies were screened using molecular methods for a range of honeybee and bumblebee pathogens. The bumblebee parasites *Apicystis bombi*, *Crithidia bombi* and *Nosema bombi*, and the honey bee parasites *Nosema ceranae*, *Nosema apis*, Deformed Wing Virus and *Ascosphaera* were all detected in either bees or their pollen. The infectivity of the parasites was confirmed by a series of infection experiments with *Bombus* and *Apis* workers, and *Apis* larvae. Exposure to the pollen or faeces of commercial bumblebees caused lethal and sub-lethal effects on treated bees. Given the geographic scale of the commercial bumblebee trade, it is inevitable that non-native strains of parasites are being distributed to new regions. Pathogen spillover from commercial bumblebees represents a clear threat to both wild bumblebees and managed honey bees.

Impact of combined pesticide exposure on individual- and colony-level traits in bees

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As the global human population continues to grow, food security becomes an increasingly pressing issue. Pesticides are essential to maintain high crop yields, but have also been implicated in causing declines in insects essential for crop pollination [1, 2]. Bees contribute ~80% of insect pollination so understanding and mitigating the causes of their current decline is imperative [3]. Our investigation had two objectives that have received little previous attention. Firstly, to understand the potential detrimental impact when bees are simultaneously exposed to field-level concentrations of two pesticides - a situation typically encountered when foraging on crops in the field [4, 5]. Secondly, to reveal the essential link between pesticide effects on individual behaviour and how these individual effects might be amplified to affect the colony as a whole. Eusocial bee colonies depend on the collective performance of numerous individuals (workers), thus whilst field-level pesticide concentrations may have only a subtle/sublethal effect on the individual [2], this could result in severe cumulative effects on the colony. We investigated the effect(s) of field-level pesticide exposure on the bumblebee *Bombus terrestris* (n = 40 colonies) including examining natural foraging behaviour using radio frequency identification (RFID) tagging (n = 259 foragers performing 8751 foraging bouts). We found that exposure to two pesticides (a neonicotinoid and pyrethroid) impaired worker foraging behaviour and increased worker mortality which had a significant impact on overall colony success. Moreover, such detrimental effects were more severe when exposed to both pesticides combined. Given that insect pollinators are often exposed to both pesticide classes in the field [6], our findings are concerning - especially for honeybees which also have a complex social organisation and depend on a critical threshold of workers performing efficiently to ensure colony survival.

Honeybee workers' reproduction and their life expectancy

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It is well known that honeybee workers in a colony without a queen and brood start laying their own unfertilized male-determined eggs. It is also known that laying workers usually come from among younger hive bees and not older foragers. We hypothesize that not the workers age but their life expectancy is the main factor causing workers reproduction. To test this prediction we used three colonies of bees (*Apis mellifera*) with two groups of workers: untreated control and the bees whose life expectancy was shortened by puncturing their thorax. Each of these groups contained eight age classes of workers marked with different colours and recruited from newly hatched workers in three-day intervals.

On the day when the youngest classes of workers hatched all the colonies were de-queened and left without a queen for 15 days. After this time marked workers from all age classes (15 to 36 days old), were collected, frozen and successively dissected. Workers' readiness to lay eggs was assessed by diameter of their ovarioles in their ovaries and by size of acini in their hypopharyngeal glands. We stated that the workers from all control age classes lived longer than injured workers from relative classes. According to our predictions the diameter of ovarioles in all workers decreased with their age, however, this parameter in control and injured bees differed significantly when they were young. Hypopharyngeal glands of control workers were also less developed in older workers while in injured ones they were weakly developed independently of their age. Our results show that life expectancy and not real age decides about females' reproductive cessation, what suggests that it is the by-product of workers' aging and their division of labour.

Behavioural reversion in honey bee workers with different life expectancy

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The age-related division of labour in a honey bee colony lies in the fact that the workers usually participate first in safe inside nest tasks and then switch to risky outside nest foraging. However, honeybees show great plasticity during their behavioural development, with their hive-to-field transition being accelerated, delayed, or even reversed in response to changing in colony conditions. It is well known that after the removal of young bees from colony, younger foragers are more likely to revert to nursing than are older ones. According to the 'division of labour by division of risk' hypothesis, we predicted that not the worker age but their life expectancy influences the decision-making by the foragers to return to inside nest nurse activity. We have tested this prediction using three groups of honey bee (*Apis mellifera carnica*) foragers of the same age. One it was control group that were untreated and two groups of workers which had reduced life expectancy (anesthetized with CO_2 or puncturing of their thorax). The results revealed that both group of treatment workers had shorter expected lifespan compared to untreated control bees. The workers with longer expected lifespan more likely revert to nursing than the same age anaesthetized and injured workers. The experiment also showed that the behavioural reversion was accompanied by ovary development and hypertrophy of hypopharyngeal glands. These results support the tested hypothesis and suggest that division of labour in eusocial insects are a consequence of the different expected worker lifespan and the different risk associated with each task.

Members of molecular signaling pathways underling hind leg development in honeybee castes

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Beyond the physiological and behavioural characteristics, differences in appendage morphology between the workers and queens of *Apis mellifera* are pre-eminent. Some appendage specializations in the hind legs of honeybee workers, which are highly specialized pollinators, deserve special attention. The hind tibia of worker has an expanded bristle-free region used for carrying pollen and propolis, the corbicula. In queens this structure is absent. Although the morphological differences are well characterised, the genetic inputs triggering the development of this alternative morphology remain unknown. Microarray analysis detected 1952 differentially expressed genes (DEG) during workers and queens hind leg development. The gene expression signature of both castes reveals a similar pattern of genes controlling development. At the beginning of the last larval instar were detected only 15 DEG between the castes whereas at pre-pupal stage there are 97. We identified a set of genes up-regulated in workers related to sensory organ development in *Drosophila* corroborating the hypothesis that the bristles presents in the workers' hind leg are mechanosensorial. In queens, there is an up-regulated gene (GB18794), which is known to be positively regulated by the TOR pathway and part of the RNA Poll Complex. Moreover, at the beginning of the last larval instar, Ultrabithorax activators are more expressed than in prepupae and early pupae, a time when Ultrabithorax expression is approximately 25 times higher. Within the gene expression signature we identified a cluster formed by genes in which Ultrabithorax, Twist and Zeste binding sites are over-represented. Furthermore, we have shown that Ultrabithorax localization during leg development recapitulates the bristles localization in the tibia/basitarsus of the adult legs. Our findings suggest the existence of a complex dynamics at the level of mRNA transcription control driven by TOR and Hox pathways activating different set of genes and ending in caste-specific morphologies and behaviours in the honeybee.

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On the transmission strategies of the parasitic mite *Varroa destructor* among beehives.

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Among the diseases targeting honeybee hives, the parasitic mite *Varroa destructor* is thought to be a central factor contributing to the huge annual losses of honeybee colonies. Halting mite spread is therefore of primary importance to save honeybee colonies from further decline; this goal can be achieved only by deepening the knowledge on *Varroa* transmission strategies. Although, it is documented that beekeeping practices and bees robbing behaviour represent important ways of mite transmission, the natural route used by the mites to abandon a collapsing colony has not been deeply investigated so far. Previous researches have shown that mites prefer to ride on a nurse bee, recognizing it from foragers via cuticular hydrocarbons; however, this preference is advantageous for mites only when they need to be transferred to another brood cell of the same beehive to reproduce. On the contrary, in highly infested or close to collapse colonies, we would expect mites to adopt a strategy that allows them to abandon such a compromised situation by moving onto foragers. So, we explored whether, with increasing colony infestation level, mites change their preference for hosts with different tasks by using binary choice tests. Our results show that the mite strategy (to leave or to stay within the hive) depends on the level of mite infestation within the hive. We found that, at low infestation rate, mites remain within the hive and promote their reproduction by riding nurse bees. When infestation level increases, GC-MS analysis show that the chemical signatures of nurses and foragers overlap. This chemical profile homogenization between bees with different tasks does not provide mites with the cues for discrimination, promoting mites departure from infested hives by riding foragers.

Evidence for a negative correlation between the honey bee pathogens *Nosema ceranae* and Deformed Wing Virus.

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Interactions between pathogens might contribute to honey bee colony losses. We investigated if there is an association between the microsporidian *Nosema ceranae* and the deformed wing virus (DWV) in different body sections of individual honey bee workers (*Apis mellifera ligustica*). Our data provide correlational evidence for antagonistic interactions between the two pathogens in the midgut of the bees.

The APENET project: a multidisciplinary approach

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"APENET: monitoring and research in beekeeping" was a two year research project funded by the Italian Ministry of Agricultural, Food and Forestry Policies. The project approached many of the factors potentially involved in the colony losses phenomenon, including a monitoring program to assess entity of the losses and their distribution across time and space.
The research topics concerned 3 major factors: pesticides, pathogens, and environmental effects.
Research on pesticides focused on several issues related to neonicotinoid insectides (imida cloprid, clothianidin, thiamethoxam) and fipronil: the impact of seed coating and dispersal of pesticide dust during sowing (principally of maize) and possible mitigation measures; sublethal effects (impairment of learning and memory abilities); interactions with pathogens (causal agents of European foulbrood and nosemosis).
Research on pathogens revealed the genetic mechanism underlying the interaction between the parasitic mite *Varroa destructor* and the deformed wing virus (DWV) at the level of the bee immune system, explaining how latent viral infections suddenly become overt causing colony collapse.
Investigations on environmental effects included the study of genotype-environment interactions and their impact on colony vitality and the effects of nutrition on colony disease susceptibility.
Overall, the project resulted in a truly multifactorial interpretation model of the colony losses phenomenon. The interplay between factors and the potential roles in bee mortality and behaviour are graphically synthesized in the presented poster.

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