Courtship pheromones in mudpuppies and waterdogs (Necturus, Proteidae)
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Monday 28.08.2017

17.30-21.00: Registration and Welcome Reception

Tuesday 29.08.2017

8.30-9.15: Welcome and Opening Session for the CSiV XIV: Carsten T Müller & Christina D Buesching

9:20-13:00 Thematic Session I: Human chemical communication (chair: Benoist Schaal, Jan Havlícek, & Craig S. Roberts)
9.20-9.30: Introduction
9.50-10.10: Drivers of MHC-dependent mate choice in humans and primates (J. L. Abbate, J. Winternitz, E. Huchard, J. Havlíček & L. Z. Garamszegi)
10.10-10.30: Can you smell the winner? The effect of competition on human body odour (J Fialová, V Třebický & J Havlíček)
10.30-10.50: Evidence and thoughts about the influence of sex, gender and culture on human body odour perception (C. Ferdenzi, A. Fournel Arnaud & M. Bensafi)
10.50-11.20: Coffee
11.20-11.40: Olfactorily-conspicuous nipples as vital interfaces for colostrum intake in humans (B Schaal, M-L Seydel, S Doucet & K Durand)
12.00-12.20: Investigating the artificially scented ape: The impact of artificial fragrances on human olfactory communication (C. Allen, J. Havlíček, K.D. Cobey & S.C. Roberts)
12.20-12.40: Chemocommunication in a vision-ruled world (J-Y Baudouin, K Durand, A. Leleu, A Seigneuric & B Schaal)
12.40-13.00: Sexing up human pheromones: How a corporation created a myth and left a trail of false positives paved by positive publication bias (T. Wyatt)

13.00-14.00: Lunch

14:00-15:20 Open session 1 (Chair: Christina D Buesching)


14.40-15.00: Decoding Mouse Social Communication (X. Fu, Y. Yan, P. Xu, I Geerling-Vidavsky, M. Gross & T. Holy)

15.00-15.50: COFFEE

15:50-16:50 Open Session 2 (Carsten T Müller)

15.50-16.10: What may attract blue petrels to their burrows at night? (M. Gabirot, F. Bonadonna & C. T. Müller)


16.30-16.50: Effects of low environmental calcium on responses to food and predator-related odours in fathead minnows (R.S. Mirza & C. Cornell)

Pub Crawl (leaving 19:30 h)
Wednesday 30.08.2017

8.30-9.30: Plenary: Franky Bossuyt: Underwater chemistry between salamanders: Evolution of a courtship pheromone system

9:30-12:40 Thematic Session II: Proteomics (Chair: Sunita Janssenswillen)


9.50-10.10: Chemosignals in anuran breeding glands (L.M. Schulte, S. Janssenswillen, K. Roelants, S. Matthijs, P. Kok, I. Van Bocxlaer & F. Bossuyt)

10.10-10.30: A new candidate courtship pheromone in aquatically courting newt (M. Maex, D. Treer, S. Janssenswillen, I. Van Bocxlaer & F. Bossuyt)


10.50-11.20: Coffee


12.00-12.20: A preliminary study on the anal sac proteome in dogs (Canis familiaris) (S. Janssenswillen, M. Metzemaekers, P. Proost & F. Bossuyt)

12.20-12.40: Poster talks I: Proteomics

POSTER 1: Beyond sodefrin: evidence for a multi-component pheromone system in the model newt Cynops pyrrhogaster (Salamandridae) (I. Van Bocxlaer, M. Maex, D. Treer, S. Janssenswillen, R. Janssens, W. Vandebergh, P. Proost & F. Bossuyt)

POSTER 2: Seasonal changes in olfactory proteins of ewe (P. Cann, M. Chabi, C. Le Danvic, R. Lami, M. Keller & P. Nagnan-Le Meillour)

POSTER 3: Courtship pheromones in mudpuppies and waterdogs (Necturus, Proteidae) (D. Treer, M. Maex, M.A. Stoops, A. Nagel, C.J. DeChant, I. Van Bocxlaer & F. Bossuyt)

POSTER 4: A non-conventional glycosylation is involved in the coding of specific odors in domestic pig (A. Joly, S. Potelle, C. Spriet, C. Le Danvic, P. Nagnan Le Meillour)

12.20-14.00: Lunch
Thematic Session III: Olfactory Speciation

14.00-14.20: Comparative protein structure prediction and elucidation of bovine vomeronasal 1 receptor type - I (VN1R1) adopting In - silico study (Durairaj Rajesh, C. Bienboire-Frosini & P. Pageat)


15.20-16.10: COFFEE

16.10-16.30: Poster talks 2: Proteomics (cont.) & Primate olfaction


POSTER 7: Making sense of scents: Deciphering primate olfactory communication (A. Poirier)

POSTER 8: Effects of Male Axillary Extracts on the Length of Menstrual Cycle in Reproductive Age Women and Pre-Menopausal Women (V.V. Voznessenskaya & T.K. Laktionova)

16:30-17:10 Open Session 3

16.30-16.50: Social odors promote food-related preference learning through activation of the GC-D/necklace olfactory subsystem (A.D. Zimmerman, H. Zhi & S.D. Munger)

16.50-17.10: Influences of exposure to murine pheromone on the next generation (S. Koyama, H. Soini, U. Tadasu & M. Novotny)
Thursday 31.08.2017

8.30-9.30: Plenary: Peter Apps: Do mammals have a world wide web of interspecific scent signals, and could we connect to it?

9:30-10:10 Thematic Session IV: Inter-specific olfactory cues (Chair: P. Apps)


9.50-10.10: On the repellent properties of mouse urine odour to small wild-living rodents (E.V. Kotenkova, I.A. Zhigarev, V.V. Alpatov, A.O. Shchukin, V.A. Babikov & Y.A. Bazhenov)

10.10-10.40: Poster Talks 3: Inter-specific cues & Other contributions

POSTER 9: Effect of Domestic Cat Chemical Signals on Regulation of Estrous Cycles in the House Mouse Depends on Presentation Rate (T.K. Laktionova, I. Kvasha & V.V. Voznessenskaya)

POSTER 10: Preliminary results in ferret olfactory cues (Mustela putorius furo) as a predator stimulus for the house mice (Mus musculus) (P.C. Grau, J. Leclerq, E. Teruel, C. Lafont-Lecuelle & P. Pageat)

POSTER 11: Measuring lactation investment among communally nursing female house mice (A. Davidson, J.P. Green, C. De Matos Ferraz Franco, V. Lee, P Stockley, R.J. Beynon & J.L. Hurst)


POSTER 13: Taste potentiated colour biases: an alternative method for assessing taste detection thresholds in birds (H. M. Rowland)

10.40-11.20: COFFEE

11:20-13:00 Thematic Session V: Scent of Disease (Chair: Stefanie Gervasi)

11.20-11.40 LPS-induced immune system stimulation alter urinary volatiles and behavior in growing pigs (S. Devaraj, A. Rakhshandeh & J. McGlone)

11.40-12.00: Sharing an environment with sick conspecifics alters the odors of healthy mice (S. Gervasi, B.A. Kimball, M. Opiekun, T. Martin & G.K. Beauchamp)

12.00-12.20: Disease detection in human olfaction (M. Olsson)
12.20-12.40: The smell of weakness: costs and consequences of injury for social odor cues in ring-tailed lemurs (R. Harris & C. Drea)

12.40-14.00: LUNCH

14:00-15:40 Thematic Session VI: Semio-chemical Methodologies and Analyses (Chair CD Buesching)


14.40-15.00: Non-invasive sampling of body odour to investigate the information content of meerkat scent (B. M. Weiß, A. Marcillo, M. Kücklich, M. Manser, R. Holland, C. Birkemeyer & A. Widdig)

15.00-15.20: The male effect: finding a way through the jungle of chemical cues (C. Le Danvic, M. Dillenbourg, M. Keller, L. Schibler & P. Nagnan-Le Meilour)


15.40-16.00: Sampling and analysis of VOCs & SVOCs– What are the best tools for the job? (Massimo Santoro, Ilaria Ferrante, Hannah Calder)

16:00-16.40: COFFEE

16:10-15:50 Open session 4

16.30-16.50: Temporal chemical profiling of urine volatile compounds of domestic cats and their olfactory discrimination abilities (Chiharu Suzuki, Minoru Maita, Tamako Miyazaki, Tetsuro Yamashita & Masao Miyazaki)

16.50-17:10: Identification of key compounds for the individual recognition in the domestic cats (Minoru Maita, Chiharu Suzuki, Jana Caspers, Tetsuro Yamashita, Stefan Schulz & Masao Miyazaki)

19.30-10.00: Conference Dinner
Friday 01.09.2017

8.30-9.30: Plenary: Jane Hurst: Scent wars: unravelling the complexity of competitive scent signalling

9:30-10:50 Thematic Session VII: Olfactory advertisement (Chair Jane Hurst)

9.30-9.50: Urinary volatiles and bioindicators related to the reproductive status in giant panda (Ailuropoda melanoleuca) (M. Dehnhard & J. Brown)

9.50-10.10: Females prefer cooperative males based on cues different from cooperative behaviours: evidence from the Mound-building mouse, Mus spicilegus (A. Tognetti, M. Raymond, G. Ganem & C. Faurie)

10.10-10.30: Sex-steroid excretion in urine as olfactory advertisement in European badgers (Meles meles) in relation to circulating plasma titres (N. A. Sugianto, H.V. Tinnesand, D. W. Macdonald & C. D. Buesching)

10.30-10.50: Scent-marking as Honest Fitness Advertisement in European Badgers (Meles meles) (T. Allen, C. D. Buesching & D. W. Macdonald)

10.50-11.30: Coffee

11.30-11.50: Poster Talks 4: Advertisement


POSTER 16: Exploring VOCs from harbour porpoise excreta (M. Gabirot, B. Bouchard, A. Célérier, L. IJsseldijk & S. Campagna)

POSTER 17: Putative pheromone of snow leopard, Panthera uncia (Schreber, 1775) (S. Das, P.L. Das & M.P. Sarkar)

12.00-13.00: Closing session and planning of next CSiV conference

13.00-14.00: Lunch

14.30: Excursions (Cardiff Castle, Dr Who Experience)
Abstracts of Talks and Posters in order of Appearance

Tuesday 29 August 2017

Thematic Session I: Human Chemical Communication (Chair B. Schaal, J. Havlícek & C.S. Roberts)

Talk: The effect of dietary restrictions on human body odour quality

J. Havlíček\(^1\), J. Fialová\(^1\), & S.C. Roberts\(^3\)

\(^1\)Faculty of Science, Charles University, Prague, Czech Republic;
\(^2\)National Institute of Mental Health, Klecany, Czech Republic;
\(^3\)University of Stirling, Stirling, UK

Previous studies on various vertebrates have shown that quantity and quality of food intake affect odour attractiveness as perceived by potential mates. Similarly, in humans, the quality of body odour is affected by ingested foods, such as meat and garlic intake. Nevertheless, it is not known whether quantity of food has an impact on body odour attractiveness. Thus, here we tested how starvation affects the hedonic quality of human axillary odour. Odour samples (cotton pads fixed in both armpits and worn for 12 hours) were obtained from healthy female donors across three conditions: i) during their habitual food regime; ii) after 48 hours of starvation (drinking water was provided), and iii) 72 hours after restoration of caloric intake. Axillary samples were assessed by male raters regarding their pleasantness, attractiveness and intensity. We also collected blood samples for glucose level assays. The odour samples collected during starvation were rated significantly less pleasant, less attractive and more intense compared to odour samples collected after restoration of feeding. Interestingly, we also found that body odour was rated more pleasant, more attractive and less intense after restoration of food intake than prior to starvation. We also calculated differences in ratings of samples collected during starving and after starving. The increase in odour attractiveness as feeding resumed was negatively related to glucose change during the same period. Our results suggest that restoration of food intake positively influences hedonic quality of human body odour which might thus provide cues to current fitness status and metabolic efficiency.

Talk: Drivers of MHC-dependent mate choice in humans and primates

J. L. Abbate\(^1\), J. Winternitz\(^2\), E. Huchard\(^3\), J. Havlíček\(^4\) & L. Z. Garamszegi\(^5\)

\(^1\)CBGP Inra (UMR 1062 Inra-IRD-Cirad-Montpellier SupAgro), Montferrier-sur-Lez, France
\(^2\)Department of Evolutionary Ecology, Max Planck Institute for Evolutionary Biology, Ploen, Germany
\(^3\)CEFE CNRS (UMR 5175 CNRS-Université de Montpellier-EPHE), Montpellier, France
\(^4\)Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic
\(^5\)Department of Evolutionary Ecology, Estación Biológica de Doñana-CSIC, 41092, Seville, Spain
Genes of the major histocompatibility complex (MHC) in vertebrates are integral for effective adaptive immune response and are associated with sexual selection. Evidence supports MHC-based preference for diverse and dissimilar mating partners, in a range of vertebrates from fish to nonhuman primates. However, evidence from human mate choice studies has been more controversial. Here, we performed a phylogenetically controlled meta-analysis with meta-regression techniques using 58 effect sizes from 30 studies across 7 primate species in an effort to understand what processes may impact MHC-mediated mate choice in humans and non-human primates. Both human and non-human primate studies showed evidence of mate selection for MHC-diversity. In contrast, there was no general trend for MHC-dissimilarity, and for humans, effect sizes were observed for both MHC-dissimilar and similar mates. Couples primarily showed similarity preferences, suggesting the influence of socio-cultural assortative biases.

**Talk: Can you smell the winner? The effect of competition on human body odour**

J. Fialová\(^1,2\), V. Třebický\(^1,2\) & J. Havlíček\(^1,2\)

\(^1\)Faculty of Science, Charles University, Prague, Czech Republic; 
\(^2\)National Institute of Mental Health, Klecany, Czech Republic;

Previous research has suggested that some vertebrates such as rodents use odour cues to assess individuals’ competitive abilities or dominance. Similarly, it was shown that the odour of dominant males is perceived by women as more attractive and body odour collected during competition elicits higher skin conductance response compared to non-competitive context. Here, we investigated whether winning and losing a match, a marker of possible change in dominance hierarchy, will influence hedonic valence of human body odour. We employed mixed martial arts (MMA) as a model of real-life physical encounters. We collected body odour samples (sampling on cotton T-shirts for 30 minutes) from a set of MMA fighters before and after their fight on Czech Amateur MMA league. Obtained stimuli were subsequently rated a panel of raters regarding their pleasantness, attractiveness, and intensity. We found significant differences in body odour samples collected before and after the fight in individuals who lost their fight. Their body odour was rated as less attractive after the fight. In contrast, there was no change in odour attractiveness in the winners. Further, no similar pattern was observed in pleasantness and intensity. Our results show significant effect of competition outcome on perceived quality of human body odour with decreased attractiveness in losers. These results might be due to the differences in affective states which were previously shown to affect the quality of body odour.

**Talk: Evidence and thoughts about the influence of sex, gender and culture on human body odour perception**

C. Ferdenzi, A. Fournel & M. Bensafi

Lyon Neuroscience Research Center, CNRS-Inserm-University Claude Bernard Lyon 1, Lyon, France.
Although social communication is one of the major functions of olfaction in numerous species, in humans many aspects of this function remain poorly understood. There is increasing evidence that odours naturally emitted by the human body are extremely important in interpersonal relationships and that they influence mate attractiveness. However, our knowledge is only partial when it comes to the nature of the chemical compounds involved but also to the socio-cultural modulation of human olfactory social communication. While many studies have focused on androstenes as possibly relevant chemosignals, other compounds such as those produced in sex-specific amounts deserve attention. Here, I will present several studies investigating how some of the latter compounds are perceived and influence person perception, differentially in men and women. I will also discuss challenges for future research in this area, such as the understanding the origin of such sex differences, and especially how socio-cultural factors may shape them in accordance with social norms, individual’s experience and current needs. Another stimulating challenge is to use cross-cultural research to increase our understanding of how body odours influence human social interactions. To develop this idea, I will present a set of cross-cultural studies about odour-related feelings and attention, with a particular focus on aspects related with people’s odour. This will offer some thoughts on possible future directions, combining the study of cultural differences in human olfaction and the study of social communication through body odours.

**AM Coffee break**

**Talk: Olfactorily-conspicuous nipples as vital interfaces for colostrum intake in humans**  
B. Schaal, M.-L. Seydel, S. Doucet & K. Durand

Developmental Ethology and Cognitive Psychology Group, Center for Smell, Taste and Food Science, CNRS-Université de Bourgogne, Dijon, France

After birth, fragile newborns must ingest the colostrum as swiftly as possible to incorporate related benefits such as immuno-protection, growth of digestive tract, and colonization by gut microbiota. Reciprocally, females must respond timely to their offspring because any delay in colostrum transfer opens the way to predation by pathogenic bacteria. In this selective frame, females had to evolutionarily elaborate milk-delivering interfaces that both attract and boost sucking in their newborn, whereas newborns had to detect, process, and adequately react to nipples. One sensory mean by which nipples are made conspicuous to human newborns is olfaction. The nipples of lactating women are indeed endowed with areolar glands (AG) which development peaks by the end of gestation and during lactation. These areolar glands (so-called Montgomery glands) are composed of sebaceous glands coalesced with isolated lacteal acini. Two replicate studies (Schaal et al., 2006; Doucet et al., 2012) have established a relationship between the number of AG, mother-reported sucking of the infant, infant weight gain between postnatal days 1 and 3, and onset of stage-2 lactation. Here, we present a 3rd replicate study to further verify the relationships between the aforementioned variables in the 4 very first breast-feeds, with the assumption that these inaugural feeds are those which convey the most protective effects on newborns.
Among the 67 women (35 primiparous) followed up, 89.6% had 1 to 15 AG on each areola. They all accepted to complete the Infant Breast-Feeding Assessment Tool (IBFAT) after the first 4 feeds, before and after which their infants were weighed. Their lactational physiology was followed up in assessing 3 criteria of increased breast activity. It came out that the number of AG was positively related to reports of infant sucking, especially in primiparous mothers. Additionally, women having higher endowments in AG evinced shorter latency to lactation onset and their infants took more weight between birth and day 3. This study evidences significant links between AG and adaptive responses of neonates at their very first contact with the lactating breast. AG having been otherwise shown to emit secretions that are highly reactogenic to newborns (Doucet et al., 2009), we hypothesize that these effects are mediated by some potent chemical signal(s) released by AG.

Talk: Perfume preferences in human couples

S.C. Roberts1, E. Simpson1, J. Havlíček2,3 & C. Allen4

1 University of Stirling, Stirling, UK;
2 National Institute of Mental Health, Klecany, Czech Republic;
3 Faculty of Science, Charles University, Prague, Czech Republic;
4 Institute of Neuroscience, Newcastle University, Newcastle upon Tyne, UK

Several studies of human mate choice have provided evidence for disassortative odour preferences, as sometimes occurs in other species, and that expression of these preferences in women may be influenced by use of hormonal contraception. Other studies indicate that individual perfume choices are influenced by their interaction with one’s own body odour, such that choice may be critically influenced by the perception of the emergent perfume-body odour blend. In light of this, we tested two predictions regarding perfume preferences in established heterosexual couples: (1) that members of the couple should have divergent preferences for fragrances, and (2) that the magnitude of within-couple divergence in preference should be reduced by the woman’s use of hormonal contraception. To test these predictions, each man and woman in 27 couples independently assessed a series of eight fragrance accords used in modern perfumery. We found that, contrary to prediction, within-couple preferences for fragrance accords were positively, not negatively, correlated. Concordance in preference was not related to the woman’s contraceptive use, either currently or previously when the couple met; however, concordance in preference did predict aspects of relationship satisfaction in women, but not their male partners. Our results suggest that assortative preferences for odours may be more important than previously believed, and that shared odour preferences may be linked to aspects of human relationship functioning.

Talk: Investigating the artificially scented ape: The impact of artificial fragrances on human olfactory communication

C. Allen1, J. Havlíček2,3, K.D. Cobey4 & S.C. Roberts5

1 Institute of Neuroscience, Newcastle University, Newcastle upon Tyne, UK
Though once considered to be functionally anosmic there is now a growing body of evidence supporting the role of olfactory communication in humans. This research often focuses on ‘natural’ odours, requiring participants to avoid all fragranced products during odour collection. However, we argue that it is important to understand how human odours interact with artificial fragrances, and how this may affect olfactory communication, given that fragrances are currently, and historically, ubiquitous across societies and cultures. One study will be presented which investigated the impact of artificial fragrances on our ability to discriminate between the odours of individuals when they were wearing no fragrance, a fragrance of their choice, and an experimenter assigned fragrance. Our findings suggest that fragrance choice may be important, with discrimination rates being above chance in all conditions, but importantly with performance being better when an individual was wearing a fragrance of choice compared to an experimenter assigned fragrance. The second study presented examined the impact of fragrances on our ability to accurately assess mate choice relevant cues in body odour. We investigated the consistency of assessments between faces and odours, both with and without fragrance, finding that fragrances may enhance certain cues in specific individuals, potentially interfering with our ability to accurately assess these cues. The two studies together demonstrate that the impact of artificial fragrances may be more complex than originally thought, neither simply masking nor enhancing odours, with outcomes appearing to be dependent on the type of information being investigated.

Talk: Chemocommunication in a vision-ruled world
J.-Y. Baudouin, K. Durand, A. Leleu, A. Seigneuric & B. Schaal
Developmental Ethology and Cognitive Psychology Group, Center for Smell, Taste and Food Science, CNRS-Université de Bourgogne Franche-Comté, Dijon, France

One can only acknowledge that the human communication system is overwhelmingly dominated by vision and, to a lesser extent, by audition. This species-typical sensory ratio led to a strong bias in favor of research on visual/acoustic awareness in environmental and social contexts. Olfaction has been for long downgraded to a minor, relictual sense modality, the defect or loss of which being considered as almost inconsequential. The last decades of research have now clearly shown that olfaction infiltrate the regulation of all aspects of human behaviour and that anosmia incurs deep costs on the quality of everyday life, especially in self-adaptation and social exchanges. Despite this recent conceptual reversal, olfaction remains massively studied for itself, in relative isolation from the other senses. The present talk aims to summarize several studies on the effects of odor cues on various aspects of human visual behaviour. Specific effects will be reported on the impact of the odour context on the directionality and temporal dynamics of visual behaviour in social and
non-social situations in both adults and infants. Odor cues contribute to the multisensory interpretation of ambiguous visual cues, in a way to increase accurate responses. In sum, these results confirm that olfaction has a clear, although subtle, influence on visual processes, bringing another argument to dismiss the myth which obstinately defines humans as “microsmatic”.

**Talk: Sexing up human pheromones: How a corporation created a myth and left a trail of false positives paved by positive publication bias**

T. Wyatt

Dept. of Zoology, University of Oxford

A corporation interested in patenting ‘human pheromones’ for profit created a long lasting myth that has drawn in many scientists as well as the general public. I describe what went wrong and what would be needed to establish that we do have pheromones (chemical signals within a species). As humans are mammals, we may have pheromones. However, there is no robust bioassay-led evidence for the widely published claims that four steroid molecules are human pheromones: androstenone, androstenol, androstadienone, and estratetraenol. Positive results are highly likely to be false positives. Instead, we need to take the lead from Darwin and treat ourselves as if we were a newly discovered mammal, and use the rigorous methods already proven successful in pheromone research on other species.

**Lunch Break**

**Open session 1 (Chair : Christina D Buesching)**

**Talk: Pheromone of Indian Leopard, *Panthera pardus***

B. Ghosh¹,², S. Das¹, P. Das¹, S. Manna³, S. Pal Chowdhury³, M. Poddar Sarkar¹ & R.L. Bhamachary⁴

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Ethochemical characterisation of Marking Fluid (MF) and Urinary (UR) pheromone of the Indian Leopard, Panthera pardus fusca (Meyer, 1794) were carried out for last few years on some wild-born but rescued and reared under captivity in two rehabilitation centres of West Bengal (26°25′102″ N, 89°43′33.366″ E and 26°37′5.142″ N, 89°12′26.412″ E), India. We highlight three modes of ‘scent marking’ in both the sexes of leopard-direct squirting while standing erect and the tail is raised upward (MF), second squirting posture with low height-jet and keeping knees bent (MFU) and the third one normal urination (UR) with folded legs when tail almost touches the ground. Other behaviours like flehmen, cheek and body
rubbing, nail scratching, vigorous sniffing and different calls were also documented. Headspace volatiles were analysed from MF and Urine of both sexes through Solid Phase Micro-Extraction guided Gas Chromatography Mass Spectrometry (SPME-GCMS). About 25 compounds with various functional groups such as pyrrole, phenylethyl alcohol, benzaldehyde, octanl, methoxy-phenyl oxime, acetophenone etc. were identified. The elusive aroma molecule of Indian Basmati rice i.e. 2-Acetyl-1-Pyrroline (2AP) which was identified earlier in tiger was also detected from volatilome of MF of the animal. Various saturated and mono-unsaturated, poly-unsaturated and branched chain fatty acids like hexadecanoic, octadecanoic, docosanoic acid, 12-octadecenoic, 9,12-octadecadienoic, 10-methyl dodecanoic, 14-methyl hexadecanoic acid etc. were also identified from MF and UR of Leopard. The lipid containing triglycerides, wax esters, sterol esters, free fatty acids etc might act as fixatives. The amount of lipid extracted is ranged from 1-3 mg/ml.

**Talk : Infant pandas cannot discriminate between teat odors of different lactating females**

D. Liu¹, X. Xie¹, H. Wu¹, B. Luo² & G. Zhang²

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Particularly in mammals, it is vital that neonates can discriminate their own mother from other females. A wealth of studies on group living species from a broad variety of animal taxa including humans shows that infants prefer their mother’s body odor over other females; yet, studies on solitary animals are unavailable. To examine if giant panda infants can discriminate teat odors of lactating females, we first used a paradigm of habituation-dishabituation to investigate if the infants can discriminate the teat odor of their mother from that of a novel lactating female. Next, we examined if those infants prefer their mother’s teat odors to that from a novel lactating female using a two-choice test. Our results showed that the infants were habituated to their mother’s teat odor in the habituation phase \((P=0.002, n=7)\), yet show no significant increased interests (investigating, sniffing and playing with) in the teat odor of an unfamiliar lactating female \((P=0.083, n=7)\). During the two-choice test, those infants did not show significant odor preferences to either source of teat odors of lactating females. By referring to a previous study on the discrimination between mother and daughter after weaning, we primarily conclude that infant pandas may not be able to discriminate the teat odor of lactating female kin from non-kin. Further analyses of the chemical components of the teat odor by GC-MS are necessary to resolve this point unambiguously.
Mouse urine is a rich source of pheromones and other social odor cues which convey information about sex, social status, and identity; however, the molecular nature of these cues is essentially unknown. The vomeronasal organ (VNO) of mammals plays an essential role in detecting complex chemical signals that convey information about gender, strain, and the social and reproductive status of an individual. We used the responses of these neurons as an assay to identify active compounds in urine. Using a planar array of 60 extracellular multielectrodes to simultaneously record sensory responses of large numbers of vomeronasal sensory neurons (VSNs) in the isolated vomeronasal neuroepithelium (Holy et al, 2000), we isolated the spiking responses of individual VSNs during interleaved stimulation with urine samples of both genders of four strains’ (BALB/CJ, CBA/J, C57BL/6J, DBA/2J) mouse. These same urine extracts were profiled with nano LC-MS/MS to quantify the relative abundance of the individual molecular components across different urine samples. We developed a new approach, component-activity matching (CAM) (Fu et al, 2015) to select candidate ligands that “explain” patterns of bioactivity across diverse complex mixtures. Using this new approach, both previously-identified (Nordori et al, 2008) and novel sex- and strain-specific compounds were (Fu et al, 2015) or will be identified. This approach provides an unprecedented way to accelerate the identification of the molecular nature for chemical communication among mice.

PM Coffee Break

Open Session 2 (Carsten T Müller)

Talk: What may attract blue petrels to their burrows at night?

M. Gabirot¹, F. Bonadonna¹ & C.T. Müller²

¹CEFE-CNRS, Montpellier, France
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Hypogean petrels return during the summer months on remote islands to breed. The combination of nocturnal behaviours and nesting in the same burrow could allow evolution of elaborate olfactory senses in these petrels. Their strong musky odour, their anatomy and their lifestyle have led authors to suggest the important role of olfaction and plumage in social functions. Behavioural experiments showed that olfactory cues are sufficient to allow identification of their own nest. A chemical stable signature might emanate from burrow and be involved in nest recognition. However nothing is known about the origin and the
characteristics of these odours. Consequently we investigated and developed a novel robust methodology for capture, analysis and characterization of volatile organic compounds (VOCs) from nests of blue petrels. We compared chemical profiles from nests to identify VOCs keeping along years, originated from birds or not. To verify the importance of the resident in burrow on nest scents, we also extracted VOCs from feathers. Despite of chemical variations among years observed, nest odours contained social information such as species, occupancy and nest identity. Each nest seemed to have a singular chemical signature. Importantly some informative VOCs from feathers were also present in nest odours yielding a chemical signature of burrow potentially implicated in nest recognition. This work represents the first multidisciplinary case study of avian chemical communication using principally airborne signals. The elucidation of the origin, nature and function of chemical communication in birds has major eco-evolutionary implications for our understanding of avian ecology.

Talk: Harderian Gland Constituents Mediate Vomeronasal Functioning in Garter Snakes

E.J. Bentz & R.T. Mason

Department of Integrative Biology, Oregon State University, USA

The Harderian gland is the largest cephalic gland in most terrestrial vertebrates, but despite numerous studies for more than 300 years, its physiological function remains unresolved. Harderian gland secretions are diverse among different taxa and multiple lines of inquiry have resulted in many putative functions being ascribed including production of lipids, porphyrins and immune proteins. The Harderian glands of squamate reptiles are physically connected to the vomeronasal organ and solely produce the fluids in the vomeronal lumen. Protein components of these secretions are essential to vomeronal chemosensory function facilitating the detection of sex pheromones and prey kairomones, chemical signals essential to mate recognition, mate selection, and feeding. Male red-sided garter snakes (Thamnophis sirtalis parietalis) use their vomeronal organ exclusively to locate and evaluate potential mates based on female sex pheromones, whereas females do not actively search for mates. Analysis of the protein components of garter snake Harderian gland secretions via SDS-PAGE show several secreted proteins with sexually dimorphic expression patterns likely to be targets of selection and important to the natural history of these snakes. Preliminary RNA sequencing results show high expression of lipocalins – proteins which bind and solubilize lipids in aqueous solution likely acting as pheromone-binding proteins facilitating the detection of the nonpolar female sexual attractiveness pheromone. Further analyses of the protein components of Harderian gland secretions via mass
spectrometry and high throughput RNA sequencing aim to reveal the identity of these secreted proteins.

**Talk: Effects of low environmental calcium on responses to food and predator-related odours in fathead minnows.**

R.S. Mirza, Reehan & C. Cornell

Department of Biology and Chemistry, Nipissing University, Canada

Recently it has been found in areas of Northern Ontario, Western Europe and the Northeastern United States that Calcium has been declining due to decades of acid rain deposition leaching out calcium from the soil. This has led to declines in zooplankton species, and changes in invertebrate community structure. In this study, we looked at the ability of fathead minnows, Pimephales promelas, to respond to food odours, injury-released alarm cues and diet alarm cues after being acclimated to low calcium conditions (1 mg/L) for 7 days. Minnows did not increase search activity to food odours compared to the blank water control, but appeared to show a search response to both experimental and control odours. When exposed to injury-released alarm cues, minnows did not show a fright response to injury-released alarm cues under low calcium conditions. Interestingly, minnows still exhibited a fright response to chemical cues from largemouth bass fed fathead minnows, but not to cues from bass fed krill under low and normal calcium conditions (3 mg/L). Taken together, a lack of environmental calcium impairs the ability of fathead minnows to detect important ecological odours. However, some abilities are maintained after 7 days. We also gain some insight into the use of calcium within various olfactory pathways within fish brains.

**Wednesday 30 August 2017**

**PLENARY: Underwater chemistry between salamanders: Evolution of a courtship pheromone system**

F. Bossuyt

Amphibian Evolution Lab, Biology Department, Vrije Universiteit Brussel (VUB), Pleinlaan 2, B-1050 Brussels, Belgium

Newts, aquatically reproducing salamanders of the family Salamandridae, have evolutionary strongly diversified in courtship strategies. For example, while males of some species firmly hold the female in an amplexus, others have abandoned physical contact altogether and orchestrate the courtship process by tail-fanning pheromones towards the female. These
Pheromones are proteins of the Sodefrin Precursor-like Factor (SPF) family, which already had a courtship function in the earliest salamanders about 300 million years ago. Although the pheromone system essentially retained its function of enhancing female receptivity throughout salamander evolution, SPF proteins diversified through multiple gene duplications, and many newts still co-express a high phylogenetic diversity of these proteins. Here I will review how the combination of transcriptomics, proteomics and molecular phylogenetics has produced insights in SPF pheromone evolution, including divergence at speciation, evolution in relation to changing courtship strategies, and the origin of pheromones.

Thematic Session II : Proteomics (Chair: Sunita Janssenswillen)

Talk: Challenges in proteomics of the MUP multi-gene family

R.J. Beynon\textsuperscript{1}, A.J. Davidson\textsuperscript{2}, G. Gomez-Baena\textsuperscript{1}, V.M. Harman\textsuperscript{1}, L. McLean\textsuperscript{1}, M. Prescott\textsuperscript{1}, S.A. Roberts\textsuperscript{2} & J.L. Hurst\textsuperscript{1}

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Multigene families, such as the MUP cluster, encode many proteins that are very similar in their mature protein sequences. This imposes multiple challenges, both in terms of identification and quantification of different isoforms that can be addressed by proteomics. However, many proteomics approaches require the analysis of peptides derived by enzymic fragmentation of target proteins. In the case of MUPs, many such peptides are shared between multiple isoforms, rendering identification or quantification extremely challenging. Further, the MUP cluster is dynamic and there is considerable evidence for previously uncharacterised proteins in wild-derived samples, rendering the inbred mouse reference genome of limited value. In this presentation, I will discuss strategies for selective deployment of analytical approaches to isolate and quantify individual MUPs, and to produce a robust analysis of urinary MUP pattern complexity and stability. I will show how approaches on high resolution analysis of intact protein mass and charge can reveal differences in expression pattern between individuals, and confirm the stability of such patterns over time. I will also discuss rational approaches to analysis of MUP-derived peptides, and how careful analysis of these peptides can lead to robust approaches to quantification and the identification of new MUPs.

Talk: Chemosignals in anuran breeding glands

L.M. Schulte, S. Janssenswillen, K. Roelants, S. Matthijs, P. Kok, I. Van Bocxlaer & F. Bossuyt

Amphibian Evolution Lab, Biology Department, Vrije Universiteit Brussel (VUB), Pleinlaan 2, B-1050 Brussels, Belgium
Chemical signaling is an important component of the courtship display of many aquatic and terrestrial salamanders. Most known pheromones are peptides or proteins which are expressed and secreted from species-specific male breeding glands during the reproductive season to attract females or to stimulate their mating behaviours. Because anurans (i.e. frogs and toads) spend a lot of energy in acoustic and visual signaling, chemical communication during courtship has received much less attention in this amphibian order. However, anurans also have a wide diversity of sexually dimorphic glands, which due to their association with peculiar breeding behaviours and their histological similarity to salamander glands have been suggested to contain courtship pheromones as well. Because of this growing awareness that chemosignals in frogs may be more common than currently appreciated, we collected a wide range of different frog species during their reproductive seasons, covering a diverse selection of breeding gland types and combined histology with transcriptomics, proteomics and molecular phylogenetics to search for pheromone candidates. Our analyses show highly different expression patterns for different types of glands, but indicate that several of them may be associated with courtship behaviours in certain species. Our study thus further supports a role for pheromone containing breeding glands in chemical communication during anuran courtship.

Talk: A new candidate courtship pheromone in aquatically courting newts.

M. Maex, D. Treer, S. Janssenswillen, I. Van Bocxlaer & F. Bossuyt
Amphibian Evolution Lab, Biology Department, Vrije Universiteit Brussel (VUB), Pleinlaan 2, B-1050 Brussels, Belgium

Males of advanced salamanders (Salamandroidea) are unique among vertebrates in having internal fertilization without copulation. Instead, sperm is transferred via an external spermatophore, which the female takes up with her cloaca. Since a successful transfer of sperm heavily relies on a female following the male track at spermatophore deposition, males have evolved various courtship displays to persuade a female in picking up the sperm. In several aquatically reproducing newts, males do not longer display physical contact during courtship, but instead rely on emitting chemosignals to the female. Previously, we isolated and experimentally tested protein pheromones of the Sodefrin Precursor-like Factor (SPF) family and demonstrated that they can elicit female courtship responses in a European newt. Here we combined proteomic analyses with transcriptome analyses (RNASeq) of the pheromone-producing gland to show that several European newt species additionally secrete multiple 15 kDa proteins in the water during courtship. These cysteine-rich proteins do not show any apparent relationship to other known proteins in vertebrates and are, similar to SPF, among the most highly expressed proteins in the pheromone-producing glands. This high expression, together with abundant secretion during tail-fanning in water, suggests that the 15 kDa proteins serve a courtship pheromone function in newts.
Altogether, our study presents a set of promising pheromone candidates that will be studied in future behavioural experiments.

**Talk: Molecular complexity of scent signals in the Norway rat**

G. Gómez-Baena¹, K. C. Pounder², J.O. Halstead², S.D. Armstrong¹, M. Prescott¹, S.A. Roberts², L. McLean¹, J.L. Hurst² & R.J. Beynon¹

¹ Centre for Proteome Research, Institute of Integrative Biology, University of Liverpool, Crown Street, L69 7ZB, Liverpool, United Kingdom;
² Mammalian Behaviour and Evolution Group, University of Liverpool, Leahurst Campus, Neston, United Kingdom

Chemical communication in rodents is mediated by signals deposited in the environment via urinary scent marks. We performed a detailed molecular characterization of naturally voided urine and deliberate scent mark deposits in Rattus norvegicus, including samples from rats of the laboratory strains Wistar Han and Brown Norway and also wild individuals. Our results reveal that the protein compositions of rat urine and scent marks differ significantly. In the urine of male rats, the main protein components are isoforms of the major urinary protein (MUPs) system, a family of lipocalins of ~18 kDa which have been shown to play a central role in chemical communication in mice. Comprehensive analysis of the MUP system in male rat urine indicated that some isoforms can be phosphorylated. In male scent marks the main protein constituents originate from the ventral prostate, notably prostatein and cystatin related protein, along with a complex mixture of N- and C-terminal truncated forms of MUPs (~17 kDa in size). Analysis of male accessory glands traced the origin of the truncated MUPs to the preputial glands. In females, MUPs are absent from urine, but are the main components in the protein fraction of female scent marks. Characterization of extracts from clitoral glands, the female equivalent of the male preputial glands, identified these as the source of MUPs in female scents. These MUPs are also truncated at the N- and C-terminal extremities, as found in male scents. Our data reveal an unexpected complexity in the molecular composition of scent marks in Rattus norvegicus. This gives rise to new hypotheses about the role of the different components in chemical communication in the Norway rat.

**AM Coffee Break**

**Talk: Impact of male effect and anoestrus oestrus statuses on goat olfactory secretome**

M. Chabi¹², C. Le Danvic³, M. Chasles¹, M. Keller¹, P. Nagnan-Le Meillour²

¹ UMR PRC INRA ;
² UGSF-UMR 8576-USC INRA ;
³ ALLICE

It is reported in ungulates (goat and sheep) that an active male put in contact with anoestrus females can induce a quick change in female LH secretion, which triggers female sexual
activity reactivation (Chasles et al. 2016). This “male effect” can be mimic by male odours. Those chemical signals are detected by the female thanks to odorant receptors (OR). To bring odorants to the OR, Odorant-Binding Proteins are required (OBP). OBPs are small proteins secreted in nasal mucus proposed to insure odorant solubilisation and to give its recognition specificity for the different animal species. Those proteins have been shown to bear post-translational modifications (PTM) including O-GlcNAcylation and phosphorylation in pig, for the first time in 2014 (Nagnan-Le Meillour et al., 2014). Unlike pigs, goats display a seasonal reproductive activity, and thus get two statuses: anoestrus and oestrus but are those statuses characterized by specific OBP modification? In addition, if the male odour gets the capacity to induce LH secretion in female, does the male effect induce any PTM changes on OBPs in goats? To answer these questions, we investigated for the first time the goat olfactory secretome at different reproduction statuses. We used 2D-DIGE (2-dimensional differential electrophoresis) coupled to high-resolution mass spectrometry to compare olfactory secretomes of anoestrus, oestrus females and females exposed to an active male bouquet. Those methods allowed us to describe for the first time the goat OBPs involved in the reproduction process and highlight the putative PTM implication.

**Talk: Identification and characterization of a seasonally-expressed sexually dimorphic urinary lipocalin in the bank vole *Myodes glareolus***

G.M. Loxley¹, J. Unsworth¹, M. Turton¹, A. Davidson², A. Jebb², J.L. Hurst² & R.J. Beynon¹

¹Centre for Proteome Research, Institute of Integrative Biology, University of Liverpool, UK; ²Mammalian Behaviour and Evolution Group, Institute of Integrative Biology, University of Liverpool, UK

The expression of high levels of protein in urine is common in some groups of rodents, providing important signals in scent as well as providing a slow release of volatile ligands bound to the protein. Rats & mice (muroid rodents) release abundant levels of MUPs that exhibit high levels of polymorphism. Voles (cricetid rodents) on the other hand display simpler protein profiles, but the predominant proteins are lipocalins related to MUPs, generically termed odorant binding proteins. In this study, we characterized a seasonally-expressed, male-specific urinary lipocalin in the bank vole, *Myodes glareolus*. The protein sequence, obtained by peptide mass spectrometry predicted a protein that was exactly consonant with the observed mass of the protein. Further, leucine/isoleucine ambiguity was resolved by metabolic labelling, with dietary deuterated leucine. The protein is a lipocalin, most closely related to aphrodisin and odorant binding proteins (OBPs), with a clearly defined central cavity. However, it is distinct from three urinary proteins previously identified in this species, which are present at much lower levels. Homology modelling revealed two equally valid conformations that altered the accessibility of the central binding cavity, suggesting a role for ligand binding and release. The presence of the switchable
binding pocket, combined with the seasonal and male-specific expression pattern leads to the presumption that this protein is involved in sexual chemical communication in bank voles.

**Talk: A preliminary study on the anal sac proteome in dogs (Canis familiaris)**

S. Janssenswillen¹, M. Metzemaekers², P. Proost² & F. Bossuyt¹

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Dogs mark their environment with scent signals to inform conspecifics about their individual status. Anal sac glands are known to play an important role during this chemical signalling. So far, small volatile molecules and bacterial flora have been identified from these gland secretions. However, anal sacs also secrete large quantities of nonvolatile proteins, which until now have remained uncharacterised. Here we examined the anal sac secretions of twenty domestic dogs. To investigate whether an individual’s status is reflected in the anal sac proteome, excretion patterns were compared among genders, ages, breeds, and heat cycle times. Additionally, we assessed the impact of neutering on scent marking.

Comparative proteomics and sequencing showed differential excretion of a 17kDa protein that we identified as an Odorant Binding Protein (OBP), a member of the lipocalin protein family. In other vertebrates, OBPs serve as pheromones and as odorant/pheromone carriers. Screening of the dog genome reveals that the corresponding obp gene is part of a cluster of three related genes, one of which encodes Can f4, an OBP previously found to be abundant in saliva and dander. We found that the anal sac OBP reaches high concentrations in puppies and young dogs, but not in adults. This finding suggests that this protein has a function in chemical communication during the first years of a dog’s life, which may be lost when raised in household situations. Our preliminary data show that proteome analyses can provide valuable information for further unravelling the chemical mechanisms of scent signalling in Canidae.

**Poster talks I : Proteomics**

**POSTER 1: Beyond sodefrin: evidence for a multi-component pheromone system in the model newt Cynops pyrrhogaster (Salamandridae)**

I. Van Bocxlaer², M. Maex², D. Treer², S. Janssenswillen¹, R. Janssens², W. Vandebergh¹, P. Proost² & F. Bossuyt¹

¹Amphibian Evolution Lab, Biology Department, Vrije Universiteit Brussel (VUB), Pleinlaan 2, B-1050 Brussels, Belgium;
Sodefrin, a decapeptide isolated from the male dorsal gland of the Japanese fire belly newt Cynops pyrrhogaster, was the first peptide pheromone identified from a vertebrate. The fire belly salamander and sodefrin have become a model for sex pheromone investigation in aquatically courting salamanders ever since. Subsequent studies in other salamanders identified SPF protein courtship pheromones of around 20 kDa belonging to the same gene-family. Although transcripts of these proteins could be PCR-amplified in Cynops, it is currently unknown whether they effectively use full-length SPF pheromones next to sodefrin. Here we combined transcriptomics, proteomics and phylogenetics to investigate SPF pheromone use in Cynops pyrrhogaster. Our data show that not sodefrin transcripts, but multiple SPF transcripts make up the majority of the expression profile in the dorsal gland of this newt. Proteome analyses of water in which a male has been courting confirm that this protein blend is effectively secreted and tail-fanned to the female. By combining phylogenetics and expression data, we show that independent evolutionary lineages of these SPF’s were already expressed in ancestral Cynops species before the origin of sodefrin. Extant Cynops species continue to use this multi-component pheromone system, consisting of various proteins in addition to a lineage-specific peptide.

POSTER 2: Seasonal changes in olfactory proteins of ewe

P. Cann¹, M. Chabi¹, C. Le Danvic², R. Lami R³, M. Keller⁴ & P. Nagnan-Le Meillour¹

Sheep species displays a seasonal breeding characterized by a period of sexual activity followed by a period of sexual rest. During the female anoestrus, the perception of the odour of a sexually active ram induces reactivation of ewe gonadotropic axis, leading to oestrus. Changes in olfactory system have been reported according to this switch, but more likely at central nervous steps than at peripheral level of odours detection, partly realised by odorant-binding proteins (OBP). We suggest that in sheep, the olfactory secretome is mainly composed of OBP isoforms, as it was demonstrated in pig species (Nagnan-Le Meillour et al., 2014), and that its composition could vary according to the physiological status of the animal. Besides, it has been shown that the presence of bacteria decreases the level of transcripts encoding OBPs in rat (François et al., 2016). So, the aim of our study is to compare ewe olfactory secretome and bacteriome between the anoestrus and oestrus periods. Proteins extracted from nasal mucus of two ewes were analysed by 2-dimensional electrophoresis followed by Mass Spectrometry analysis (MALDI-TOF/TOF and Nano-LC-MS/MS) and compared between the two sexual periods. We report here the first identification of ewe olfactory secretome and its variation between oestrus and anoestrus periods. Our results comfort the hypothesis that the sensory equipment of ewe is under
control of its physiological status, some proteins being specifically expressed at oestrus time, possibly to increase the detection of sexually active ram odour.

**POSTER 3: Courtship pheromones in mudpuppies and waterdogs (Necturus, Proteidae)**

D. Treer¹, M. Maex¹, M.A. Stoops², A. Nagel², C.J. DeChant³, I. Van Bocxlaer¹ & F. Bossuyt¹

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Mudpuppies and waterdogs (genus *Necturus*) are relatively big, paedomorphic salamanders that inhabit rivers and lakes of North America. Together with the olms (*Proteus anguinus*), their blind European relatives, they are the only extant representatives of the family Proteidae. Necturus species are characterised by longevity and a prolonged time for reaching sexual maturity. They reproduce by internal fertilisation, in which females pick up spermatophores deposited by the males in the environment. Because several internally fertilising salamander families are known to use pheromone communication to optimise their reproductive strategy, we studied the courtship behaviour of Necturus species to identify behavioural patterns related to pheromone transfer. Our observations revealed that both sexes wag their tail in proximity of an individual of the opposite sex. This behaviour, in combination with the fact that these species possess cloacal glands that are homologous to pheromone glands of other salamander families, indicates that chemical communication plays an important role during courtship. We then combined transcriptomics of the pheromone glands, proteomics of courtship water and molecular phylogenetic analyses to further identify candidate pheromones in this urodelan lineage. Our study fills an important gap of information on pheromone use in an approximately 150 million year old evolutionary lineage of salamanders.

**POSTER 4: A non-conventional glycosylation is involved in the coding of specific odors in domestic pig**

A. Joly, S. Potelle, C. Spriet, C. Le Danvic, P. Nagnan Le Meillour

Unité de Glycobiologie Structurale et Fonctionnelle (UGSF), UMR8576, CNRS/Université de Lille 1/USC INRA1409 cité scientifique, bâtiment C9 75655 Villeneuve d’Ascq CEDEX

In mammals, Odorant Binding Proteins (OBPs) are small soluble and secreted proteins that bind and solubilize odours and pheromones, prior to interaction with olfactory receptors (ORs). The nature of the ligand of ORs is still unknown and could be either the odorant compound itself or the complex formed by the binding between the OBP and the odorant. The later hypothesis implies a larger OBP diversity than the 2–3 gene products identified in each species. Nevertheless, we identified about thirty isoforms of OBP in the pig olfactory secretome by two-dimensional electrophoresis coupled to high-resolution mass...
spectrometry (Nagnan-Le Meillour et al., 2014). These isoforms are generated by post-translational modifications (PTMs): phosphorylation and O-β-N-acetylglucosaminylation (O-GlcNAcylation). This later PTM is usually described for nuclear and cytoplasmic proteins where the addition of GlcNac on serine and threonine residues is catalyzed by a single O-GlcNAc transferase (OGT). However, OBPs are secreted proteins for which there are only a few reports of extracellular O-GlcNAcylation. Recently, an enzyme called EGF-domain repeats-N-acetylglucosaminyltransferase (EOGT) was evidenced in Drosophila to perform O-GlcNAcylation of extracellular domains of transmembrane proteins. To figure out if O-GlcNAcylation of porcine (Sus scrofa) OBP is catalyzed by porcine EOGT (Nagnan-Le Meillour et al., 2014), we produced EOGT in two different expression systems to analyze its enzymatic activity in vitro with its two putative substrates, GlcNAc and OBP. We also performed an in cellulo analysis of OBP O-GlcNAcylation by a gene rescue method in HeLa cell line. We also monitored EOGT trafficking by a live cell-screening system.

Lunch Break

Thematic Session III: Olfactory Speciation

Talk: Comparative protein structure prediction and elucidation of bovine vomeronasal 1 receptor type - I (VN1R1) adopting in-silico study

R. Durairaj, C. Bienboire-Frosini & P. Pageat

Research Institute in Semiochemistry and Applied Ethology

The perception of putative pheromones was mediated by vomeronasal 1 receptors (V1R) of G-protein coupled receptor (GPCR) superfamily proteins in the accessory olfactory epithelium (AOE). Also, the V1R located in the nasal cavity and interconnecting large olfactory sensory neurons directed by multigene superfamily in most species. In bovine, the V1R is crucially involved in chemical communication related to reproduction and pheromone transport activity. However, the structure modeling and annotation of bovine V1R has not yet been reported. In this present study, the sequence analysis, structure prediction and function elucidation of bovine V1R made using computational methods. The results revealed that: (i) the highest proportion of α-helix, strand, and random coil in secondary structure of V1R; (ii) Comparative structure prediction of V1R with various homolog templates using Ab initio modeling; (iii) Validation of structure showed 98.6% of most favored regions with allowed regions; (iv) Structural superimposition, topology and transmembrane prediction were additionally supported the structural modeling of bovine V1R using computational analysis. Thus, for the first time demonstrated the comparative structure prediction of bovine V1R facilitate to predict the putative isoforms of pheromones involved signal transduction in reproduction.
Talk: A novel lipid metabolism system developed in cats produces a species-specific chemosignal

M. Miyazaki, A. Futsuta, W. Hojo, T. Miyazaki & T. Yamashita

Iwate University, JAPAN

Many mammals secrete species-specific chemicals that function as signals for conspecific recognition. Previous studies identified species-specific chemicals in several species. For example, domestic cats excrete 2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid known as felinine in cat urine. Felinine is a precursor of volatile 3-mercapto-3-methylbutanol, which is a major contributor to cat urinary odor. However, little is known regarding how cats evolutionally acquired their biosynthetic systems. Here we show a previously unknown metabolic pathway that develops in domestic cats and produces a cat-specific chemosignal. The increased isomerase levels in the mevalonate pathway for isoprenoid/cholesterol biosynthesis develop a unique pathway from a cholesterol biosynthesis intermediate to electrophilic alcohol, which is conjugated to glutathione. The novel glutathione-conjugate is ultimately metabolized to felinine in the cat urine. Since the modified mevalonate pathway regulates the glutathione-conjugate and its metabolite levels, but not serum cholesterol levels in cats fed a high-fat diet, this modification excels to prevent biosynthesis of excess cholesterol in obligate carnivore cats. These findings improve our understanding of both the lipid metabolism of obligate carnivores and the biosynthetic mechanisms of species-specific compounds for scent communication.

Talk: Olfactory speciation in Eurasian badgers Meles spp.: Diversity of subcaudal chemicals and behavioural experiments


1 CARNECCO, Tokyo University of Agriculture and Technology; 2 WildCRU, University of Oxford; 3 Trakia University; 4 Daido Bunseki Research Inc; 5 Shikoku Institute of Natural History; 6 Japanese Ministry of the Environment

Evolutionary theory predicts members of distinct (sub-)species to be able to recognize each other, resulting in speciation. However, surprisingly few studies attempt to test this theory. Here, we use badgers (Meles spp.) as a model to investigate if their odour profiles reflect population- and species differences between 2 populations of Japanese, M. anakuma, and European badgers, M. meles. All badgers possess a subcaudal gland, unique to this genus. We posit that the general composition of badger Meles spp. secretions remain similar over geographic distances and evolutionary time, encoding genus, but that odour-profiles between M. meles and M. anakuma differ sufficiently to allow species discrimination, and
geographically-discrete populations of each species will vary within species-specific parameters. We analysed 41 adult badger sub-caudal gland GC-MS profiles from 2 *M. meles* (UK, Bulgaria) and 2 *M. anakuma* (Tokyo, Kochi) populations. Between 24—32 chemical components were identified in each profile, 4 compounds were present in each individual, and 10 compounds were present in both species. Statistical analyses revealed significant differences between the two species (PERMANOVA, p<0.05) as well as between subpopulations of each species (PERMANOVA, p<0.05). Behavioural scent-provisioning experiments on a captive adult female Japanese badger revealed that these statistical differences are biologically relevant with the animal sniffing scent from other *M. anakuma* significantly more often than scent from *M. meles*.

**Talk: Odour dialects among wild mammals**


School of Biosciences, Cardiff University, Cardiff, UK

Across multiple taxa, population structure and dynamics depend on effective signalling between individuals. Among mammals, chemical communication is arguably the most important sense, underpinning mate choice, parental care, territoriality and even disease transmission. There is a growing body of evidence that odour signals carry genetic information that may confer considerable benefits including inbreeding avoidance and nepotism. To date, however, there has been no clear evidence that odours signal population-level information in wild mammals. Here we demonstrate for the first time the existence of ‘odour dialects’ in genetically distinct mammalian subpopulations across a large geographical scale. We found that otters, *Lutra lutra*, from across the United Kingdom possess sex and biogeography-specific odours. Subpopulations with the most distinctive odour profiles are also the most genetically diverse but not the most genetically differentiated. Furthermore, geographic distance between individuals does not explain regional odour differences, refuting other potential explanations such as group odour sharing behaviour. Differences in the language of odours between subpopulations have the potential to affect individual interactions, with clear implications for reproduction and gene-flow. Odour dialects thus provide a potentially important mechanism governing mammalian meta-population structure across large landscapes.

**PM Coffee Break**

**Poster talks 2: Proteomics & Primate olfaction**

**POSTER 5: Quantitative Profiling of Major Urinary Proteins in Wild Mice**

M.C. Prescott¹, G. Gómez-Baena¹, V.M. Harman¹, A.J. Davidson², S.A. Roberts², J.L. Hurst² & R.J. Beynon¹
The Major Urinary Proteins (MUPs) are beta-barrel lipocalins of 18-19kDa that are involved in rodent communication. MUPs encode owner identity and elicit the transport and slow release of volatile molecules. One MUP, darcin, is a male pheromone that induces long term remembered attraction to the owner of a scent mark and his location. Individual wild mice express different MUP profiles, requiring rapid analytical approaches to obtain first-pass profiling of MUP content. The profile of MUPs can be analysed in two physicochemical dimensions using mass spectrometry (MS) and isoelectric focusing (IEF). Analysis of the electrospray ionisation (ESI) mass spectrum of intact MUPs defines the pattern of proteins separated by differences in mass and returns a relative quantification of the components. For absolute quantification in mass spectrometry, standards are required. We have used synthetic protein standards (QconCATs) that are produced by artificial gene design. The QconCATs are produced in bacteria and simultaneously labelled with stable isotopes. Adding QconCATS to a natural MUP mixture, followed by digestion using endopeptidases, co-generates quantotypic peptide standards (Q-peptides) alongside naturally derived analyte peptides. When analysed by liquid chromatography-mass spectrometry (LC-MS) absolute quantification of MUPs in complex samples can be achieved by reference to the labelled, mass-shifted standards. We have also evaluated an automated IEF system (Protein Simple nanoPro1000) as an orthogonal method for quantitative MUP analysis, with specific detection using a MUP antibody. We will present examples of the application of intact mass ESI-MS, QconCAT with LC-MS and IEF for quantitative MUP analysis in wild mice.

POSTER 6: Identification and characterization of a seasonally-expressed sexually dimorphic urinary protein in two species of Malagasy mouse lemurs, Microcebus murinus and Microcebus lehilahytsara

G. Loxley¹, A. Trester², J. Unsworth¹, G. Gomez-Baena¹, U. Radespiel², E. Zimmermann², J.L. Hurst¹, R.J. Beynon¹

¹ Institute of Integrative Biology, University of Liverpool, UK; ² Institute of Zoology, University of Veterinary Medicine (TiHo), Hannover, Germany

Communication via sensory cues governs reproduction and survival in primates. Malagasy lemurs belong to the basal primate group Strepsirrhini, exhibiting a rhinarium and a functional vomeronasal organ, supporting importance of chemosignalling in social communication. Lemurs display a seasonal reproduction pattern. Urinary proteins play an increasing number of roles in communication in rodents. Proteinuria in primates is usually thought to be a sign of renal dysfunction. Our aim was to explore whether primate urinary proteins are linked to sex, season and reproduction, and may thus potentially be involved in chemosignalling. Urine samples of Malagasy mouse lemurs were collected in and out of the breeding season, from male and female adults. An abundant protein was evident in some male urine samples, but limited to the breeding season. Further characterization identified
this as a WAP four-disulphide core domain protein 12 (WFDC12), homologous to anti-microbial and anti-peptidase proteins. This protein was quantifiable in individuals from all groups; in breeding season males, however, there was a 70-fold average increase in abundance compared to all other males. A strong sex-specific difference was visible, with a 90-fold increase even in low-WFDC12 males in the breeding season compared to all females. A number of other proteins revealed similar increases in abundance in this same subset of males from their homologous sequences in other primates, including semenogelin II, a seminal vesicle protein that increased on average by 4000-fold. This relationship between an increased expression of semenogelin II and WFDC12 remains to be defined. We establish bio-essays to assess any semiochemical role of the WFDC12 protein.

**POSTER 7: Making sense of scents: Deciphering primate olfactory communication**

A. Poirier
Anglia Ruskin University, Cambridge, UK

Olfactory communication is an important mediator of social interactions in mammals, providing information about an individual’s identity and current social, reproductive, and health status. This research project uniquely combines behavioural and chemical information, in both wild and captive conditions, to decipher some aspects of olfactory communication in callitrichines (marmosets and tamarins, New World primates). The study explores how species, social, and individual characteristics, but also environmental conditions (i.e. captive vs. wild conditions), might influence patterns of callitrichine scent-marking behaviour, as well as the chemical composition of scent-mark secretions. Behavioural observations, as well as swabs of scent-glands and naturally deposited scent-marks, were collected from captive groups of marmosets and tamarins in several UK zoos. Additionally, swabs of scent-glands were obtained from a wild population of tamarins in the South-eastern Peruvian Amazon as part of an annual trap-and-release program, and compared with those collected in captive conditions. Chemical samples were analysed using headspace gas chromatography-mass spectrometry. This study intends to provide baseline knowledge applicable to captive husbandry practices, including breeding of rare species.

**POSTER 8: Effects of Male Axillary Extracts on the Length of Menstrual Cycle in Reproductive Age Women and Pre-Menopausal Women**

V.V. Voznessenskaya & T.K. Laktionova
A.N.Severtsov Institute of Ecology & Evolution, Russian Academy of Sciences

Human underarm secretions, when applied to women recipients, alter the length and timing of the menstrual cycle (Preti et al., 2003). Specific aim of our study was to investigate the influence of male axillary extracts on the length of menstrual cycle of premenopausal women in comparison with women of reproductive age. Total of 43 women, age of 21–51, participated in our study. We monitored length of the menstrual cycle for each test subject.
for 4 months before experiment and followed monitoring for 2 months after the experiment. We applied axillary extracts/or diluent on the upper lip 3 times a week for 6 hours in the morning for duration of 9 weeks. Women of 21-44 years old were subdivided into three groups: with menstrual cycle length of 26-32 days (1); menstrual cycles shorter than 26 days (2) and with cycles > 32 days (3). Data from women of > 45 years old were analyzed separately. Male axillary extracts (MAE) did not affect significantly length and regularity of menstrual cycle in women with normal and regular cycles (n=12, p> 0.1). At the same time we observed significantly shorter menstrual cycles (n=12, p< 0.01) under MAE applications in women with cycles longer that 32 days. For women with menstrual cycle length < 26 days we also did not observe statistically significant changes under MAE treatment (n=9). In premenopausal women MAE applications caused significant shortening of the menstrual cycle (n=10, p< 0.01). Also we observed a tendency for more regular cycles for this group of test subjects.

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Open Session 3

Talk: Social odors promote food-related preference learning through activation of the GC-D/necklace olfactory subsystem

A.D. Zimmerman1,2, H. Zhi1,2 & S.D. Munger1,2,3

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Individuals from several diverse mammalian species can transmit dietary preferences to peer conspecifics and help animals to avoid potentially toxic foods. This “social transmission of food preference” (STFP) requires concurrent detection of one of several semiochemicals (CS2 present in the breath, or guanylin peptides excreted in feces or urine) and odors associated with a particular food source. These semiochemicals stimulate a specialized olfactory subsystem in mice that include olfactory sensory neurons (OSNs) expressing the unique olfactory receptor GC-D (guanylyl-cyclase D). GC-D+ OSNs project to the caudal main olfactory bulb, a poorly defined region that includes the “necklace glomeruli” (a target of GC-D+ OSNs) and that also receives input from other chemosensory neurons that utilize cGMP-mediated transduction mechanisms. Recent published and unpublished studies of STFP in mice will be discussed, including a dissection of the molecular mechanisms employed by GC-D+ OSNs to detect CS2 and guanylin peptides; tract tracing and activity mapping of neural circuits associated with the GC-D/necklace subsystem; and behavioral analyses of olfactory sampling and consumption in the context of this associative learning paradigm. Supported by NIDCD grant DC005633.
**Talk: Influences of exposure to murine pheromone on the next generation**

S. Koyama\(^1\), H. Soini\(^2\), U. Tadasu\(^3\) & M. Novotny\(^2\)

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\(^2\)Indiana University, Dept. Chem., Bloomington, IN U.S.A;
\(^3\)Obihiro University of Agriculture and Veterinary Medicine

The current understanding of the activity of mammalian pheromones is that endocrine and behavioural effects are limited to the exposed individuals. Here, we demonstrate that the nasal exposure of female mice to a male murine pheromone stimulates expansion of mammary glands, leading to prolonged nursing of pups. Subsequent behavioural testing of the pups from pheromone-exposed dams exhibited enhanced learning. Sialic acid components in the milk are known to be involved in brain development. We hypothesized that the offspring might have received more of this key nutrient that promotes brain development. The mRNA for polysialyltransferase, which produces polysialylated neural cell adhesion molecule related to brain development, was increased in the brain of offspring of pheromone-exposed dams at post-natal day 10 while it was not different at embryonic stages, indicating possible differential brain development during early post-natal life. We collected milk from dams at post-delivery day 10, analyzed the milk using HPLC and 1H-NMR MALDI-TOF MS. We found that there are differences in the amount of sialic acid in the milk of pheromone exposed females compared to controls (females exposed to water and females with no treatment).

**Thursday 31 August 2017**

**PLENARY: Do mammals have a world wide web of interspecific scent signals, and could we connect to it?**

P. Apps

Botswana Predator Conservation Trust

Although nearly all the work on mammal semiochemistry has been on single species, interspecific scent marking is quite widespread, and recent camera trapping studies of carnivore guilds in Canada, the Pacific Northwest of the USA, Costa Rica and Botswana have shown carnivores using multi-species scent-marking sites. There are records of interspecific marking from at least 44 mammals, with a rapidly expanding web of known connections that make interspecific scent communication look like the rule rather than the exception. Keeping track of competitors by exchanging scent marks makes ecological sense; any individual is more likely to encounter scent marks from animals of other species than from its own, most carnivores compete for food with most of the others, and the consequences of interspecific interference competition are dire and strongly asymmetrical. This new corner of the
mammal semiochemical field presents both opportunities and challenges. We will need to understand the role of interspecific semiochemistry in ecological interactions alongside the role of intraspecific scent signals in social behaviour and reproduction. If interspecific scents’ main role is to signal merely the recent presence of a competitor they may be more accessible to being artificially recreated than are intraspecific scents with their signals of multiple reproductive and social parameters and individual signatures. On the other hand, if different species are overmarking one another, how do we ever collect a clean scent mark from the field?

**Thematic Session IV: Inter-specific olfactory cues (Chair: Peter Apps)**

**Talk: Early Olfactory Experience Affects Perception of Predator Odors in the House Mouse**

V.V. Voznessenskaya, I.G. Kvasha, T.K. Laktionova, A.B. Klinov & M.A. Klyuchnikova

A.N. Severtsov Institute of Ecology & Evolution, Moscow, Russian Federation

Unique amino acid L-felinine and its volatile derivatives may be used by the house mouse to recognize potential predators, their physiological status and may affect reproductive output in mice. Aim of our study was to examine whether early olfactory experience (EOE) of mice with cat chemosignals may affect sensitivity to target odors later in adulthood, modulate behavioral or neuroendocrine responses and whether these changes in sensitivity correlated with neural activation in olfactory bulbs. Olfactory thresholds to cat urine and L-felinine were measured with an automated olfactometer (Knosys, USA). Fecal glucocorticoid metabolites and plasma corticosterone were monitored using an ELISA technique. Behavioral patterns were analyzed using an open field paradigm (2 different modifications). We performed immunohistochemical studies to identify neural substrate involved in reception and analysis of L-felinine and derivatives. Exposures of mice to cat chemosignals (urine or L-felinine) significantly lowered the olfactory thresholds (n=10, p<0.05; n=10, p<0.01). EOE with cat urine/felinine also decreased (n=22, p<0.01) patterns of passive-avoidance behavior to cat odors and significantly elevated investigatory activity (n=22, p<0.01). At the same time corticosterone response to cat urine/L-felinine stayed unchanged (n=10, p< 0.01) indicating the innate nature of the response. We recorded specific pattern of activation in accessory olfactory bulb (AOB) in response to stimulation with L-felinine (0.05%). EOE with L-felinine (0.05%) caused significant increase in number of Fos-positive cells in AOB in response to stimulation with L-felinine (n=8, p<0.01) as well as we recorded an increase of activated area (n=8, p<0.001).

**Talk: On the repellent properties of mouse urine odour to small wildliving rodents**

E.V. Kotenkova, I.A. Zhigarev, V.V. Alpatov, A.O. Shchukin, V.A. Babikov & Yu.A. Bazhenov

Severtsov Institute of Ecology and Evolution RAS, Moscow, Russian Federation
Synanthropic mice species are common pesky organisms in human environments. While their presence is ubiquitous home owners often complain about their unique, persistent and potent odour which is derived mainly from their urine. Evolutionarily it is not favorable. It suggests that the feature is not neutral and likely should be useful for mice for other reasons. We hypothesized that such odour could have been developed during evolution as adaptation directed to protection of buildings, special ecological niche created by humans, from other rodent species. Adult individuals of outdoor species *Clethrionomys glareolus* and facultative synanthropic species *Microtus levis* avoided the arm of Y-maze scented by *M. musculus* urine in comparison with arm with water in two-choice tests. They also prefer arm scented by urine of rabbit or outdoor mouse *Mus spicilegus* in comparison with arm scented by *M. musculus* urine. In field experiments young and juvenile *Cl. glareolus* choose to enter traps with water in comparison with traps scented by urine of *M. musculus*. These results indicate repellent properties of mouse urine odour to other rodents. We speculate that olfactory cues of house mice family could prevent inhabitation of buildings by other species of rodents. Potential role of sulfur compounds in mouse urine as repellents is discussed.

Supported by Russian Science Foundation (project №16-14-10269).

**Poster Talks 3: Inter-specific Cues & Other Contributions**

**POSTER 9: Effect of Domestic Cat Chemical Signals on Regulation of Estrous Cycles in the House Mouse Depends on Presentation Rate**

T.K. Laktionova, I. Kvasha & V.V. Voznessenskaya

A.N. Severtsov Institute of Ecology & Evolution, Moscow, Russian Federation

L-felinine is a unique amino acid found in the urine of domestic cat *Felis catus*. We examined the influence of L-felinine on regulation of estrous cycles in mice. In order to monitor estrous cycle phase (ECP) we validated noninvasive estradiol assessment technique. To control ECP vaginal smears were taken and fecal estradiol levels were determined for each female on daily basis. We used 4 groups of mice (n=38) at age of 3 months: (1) continuous action of L-felinine (0.05%; 50 µl) during 12 days; (2) application of L-felinine (0.05%; 50 µl) at regular intervals for duration of two hours on daily basis; (3) spontaneous exposures to L-felinine (0.05%; 50 µl ) but the total time of exposure during 12 days was also 24 hours; (4) control (exposure to tap water). Estradiol baseline was calculated individually for each animal; concentrations above the baseline were considered as a beginning of luteal phase. The data obtained indicate that L-felinine may affect the length of estrous cycle in mice. The number of ovulations in the group 1 significantly increased (p=0.00498, n=10). It appears to be that females habituated to the continuous action of the compound. At the same time we observed decline in a number of cycling females in the group 2 (p=0.0233, n=9). For the group 3 we observed only a tendency to decrease in a number of cycling females (p=0.0578, n=9). Different modes of exposure to L-felinine produced different effect on estrous cycles in
mice.
Supported by RFBR 16-34-00872

POSTER 10: Preliminary results in ferret olfactory cues (*Mustela putorius furo*) as a predator stimulus for the house mice (*Mus musculus*)


Research Institute in Semiochemistry and Applied Ethology Apt, France

The house mice (*Mus musculus*) as other small rodents are in the base of vertebrate predator’s trophic cascades. They are the most widespread mammal on earth after humans, which along with its fast sexual cycle and prolificity means large populations and a basic source of nutrients for a wide spectrum of predators. As macrosmatic animals, mice use olfaction as a primary tool to avoid predators, however little is known about the predator olfactory cues and behavioral reactions linked to these stimuli. With this study we performed a preliminary approach to mammalian and reptilian olfactory predatory cues of the house mice. For this porpoise we carried out a choice test, where we measured during 10 minutes the total duration that mice remained in the nearby area or far end area from the predatory stimulus, mice had no physical access to the stimulus, and both parts were identical. Our results showed that mice significantly avoided ferret olfactory stimuli from fur and faeces. These results are in line with a recent study that showed avoidance of hamsters to ferret urine depending on the ferret’s diet (Apfelbach, Soini, Vasilieva, & Novotny, 2015). However further research should delve in ferret’s olfactory cues and semiochemicals as a significant rodent predator.

POSTER 11: Measuring lactation investment among communally nursing female house mice

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Wild female house mice (*Mus musculus domesticus*) nest socially and often rear offspring communally with chosen nest partners. Cooperation among communally nursing females yields important fitness benefits, including a larger number of offspring weaned and increased weight of pups. It has also been shown that females prefer to nest and rear offspring communally with sisters. Although females appear to care for offspring in communal nests indiscriminately, little is known about the lactation investment made by each mother in her own or a nest partner’s offspring because of the difficulty of measuring this. To overcome this challenge, we designed an experiment in which two communally nursing females fed on separate diets supplemented with an amino acid differentially
labelled with stable isotopes, using custom-designed feeders. As the isotope labels transfer to the offspring via each female’s milk, the ratio of these two labels allowed us to quantify the precise amount of lactation investment from each female in each individual pup in the communal nest. We established communally nesting pairs of females that were either familiar littermate sisters or unrelated and unfamiliar until they were paired as adults. This is allowing us to address whether females invest more in their own offspring than in those of another female; whether lactation investment is more egalitarian between two sisters compared to unrelated females; and whether females that give birth second (i.e. have younger pups in the communal litter) invest more in the offspring of both litters.

POSTER 12: Latrine use patterns of raccoon dogs (Nyctereutes procyonoides) in an urban area of Tokyo

M. Tsunoda1,3, Y. Kaneko1, T. Sako5, R. Koizumi1, K. Iwasaki1, I. Mitsuhashi1, M.U. Saito1, C. Newman3, D.W. Macdonald3 & C.D. Buesching3

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2 Imperial Household Agency, Japan ;
3 Wildlife Conservation Research Unit, University of Oxford, UK

Although urbanization is one of the leading threats to wildlife conservation, some species have adapted to living alongside humans. Here we used a population of wild raccoon dogs (Nyctereutes procyonoides) in the Akasaka Imperial Grounds in central Tokyo as a model to investigate how latrine using carnivores can maintain their socio-spatial organisation under human disturbance. Between 2012 -2014 we employed 4-11 camera traps positioned at a total of 12 different latrines located in different habitat types and subjected to varying levels of human disturbance, to analyse the temporal patterns of a total of 2459 latrine visits, of which 878 included defecation events. Overall, latrines disturbed by humans returned less daytime visits (with and without defecation events) than undisturbed ones and activity shifted from day- to night-time. Latrine use (i.e., visits with and without defecation events) increased towards winter, coinciding with dispersal, and showed a seasonal shift from diurnal to nocturnal usage patterns. GLMM results also confirmed that raccoon dogs defecated at open latrines more frequently than at sheltered ones. Raccoon dogs likely avoid the perceived predation risk from human disturbance by shifting their vulnerable activity (i.e., defecating) to minimise disturbance, where the inherent longevity of olfactory signals allows particularly macrosmatic carnivores to maintain their socio-spatial organisation through latrine-centred olfactory communication. We posit that these behavioural modifications may allow raccoon dogs to maintain their socio-spatial population structure even in urbanised areas that are affected by human disturbance.
Poster 13: Taste potentiated colour biases: an alternative method for assessing taste detection thresholds in birds
H. M. Rowland

Department of Zoology, University of Cambridge, UK;
Max Planck Institute for Chemical Ecology, Germany

Understanding how bird predators assess the chemical content of prey is relevant to our understanding of the adaptive significance of prey with warning colours; it is also relevant to the study of avian taste perception. Birds are often assumed to have a poor sense of taste because of the reduced number of taste buds compared to other vertebrates (~300 in birds compared to 9000 in humans). Birds also have a reduced taste receptor gene repertoire size compared to other vertebrates. For example, domestic chickens have only three bitter taste receptor genes in their genome (ggTas2r1, ggTas2r2 and ggTas2r7I) compared with 51 receptor genes in frogs. Contrary to the assumption that birds have a poor sense of taste, I show that some chemicals seem to be intrinsically aversive to birds, and that birds show concentration-dependent responses to bitter-tasting chemicals. I used a colour-bias paradigm to examine the responses of chickens to different concentrations of quinine, denatonium benzoate, quassia and caffeine. This assessment of unlearned responses to naturally occurring defensive chemicals is an alternative method for assessing detection thresholds to the forced-choice solution-consumption method.

AM Coffee Break

Thematic Session V: Scent of Disease (Chair: Stefanie Gervasi)

Talk: LPS-induced immune system stimulation alter urinary volatiles and behavior in growing pigs
S.G. Devaraj¹, A. Rakhshandeh², J. McGlone³

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Volatile compounds emanating from body secretions may act as an indicator of the health status of animals to other animals. We sought to identify urinary volatiles that might act as biomarkers indicative of health status of growing pigs. Immune system stimulation (ISS) was induced in pigs (N = 5) by i.m. injection of bacterial lipopolysaccharide (LPS; 25 µg/kg BW). Urine samples were collected before (baseline) and after LPS treatment. The urinary headspace volatile compounds were identified using Solid-phase microextraction (SPME) coupled with GC-MS. Pig eye/body temperatures were measured using infrared imaging technique, and behaviors (i.e. standing, feeding, drinking, moving, lying, sitting and active) were video-recorded and quantified 24 h before and after ISS. Data were analyzed using mixed model ANOVA examining periods before and after LPS administration with pigs serving as their own control. Pig eye/body temperature increased post-ISS (at 6 h, P = 0.03)
and correlated negatively with active behaviors ($r = -0.64$, $P = 0.048$). Relative to pre-ISS, pigs spent less time standing ($P = 0.018$), moving ($P = 0.061$) and active ($P < 0.0001$) during post-ISS period. SPME-GC-MS revealed 3.6- and 5.3-fold increases in 4-Ethylphenol ($P = 0.005$) and 3-Methylphenol ($P < 0.0001$), respectively, in post-ISS urine compared with urine collected during the pre-ISS period. In ISS pigs, behavioral activity was reduced, and excretion of urinary 3-Methylphenol and 4-Ethylphenol were increased ($P < 0.01$). Changes in behavior and volatile molecules may be used as a non-invasive method of detection of fever, illness and health in farm and possibly other animals.

**Talk: Sharing an environment with sick conspecifics alters the odors of healthy mice**

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Chemical signals convey vital information about environmental risk. It is well known that pathogen infection alters body odors. “Sickness signals” can mediate behavioral avoidance of infected animals by healthy conspecifics and accumulating evidence also suggests that exposure to sickness signals activates physiological responses of signal receivers. Sickness signals could thus have cascading effects on transmission risk by influencing host contact rates and individual susceptibility. We used an experimental approach to examine effects of exposure to odors of sick, but non-infectious individuals on the odor profiles of healthy conspecifics. We employed two types of housing setups, allowing us to directly compare responses of pairs of freely interacting or physically separated mice. In a Y-maze behavioral training paradigm, biosensor mice discriminated between odors of healthy and sick mice. Unrewarded generalization trials confirmed that biosensors also discriminated between odors of healthy mice housed with healthy partners and healthy mice housed with sick partners. We followed behavioral discrimination assays with chemical headspace analyses employing gas chromatography/mass spectrometry. Chemometrics supported patterns observed in bioassays; the composition of odors of healthy mice housed with sick conspecifics was statistically distinguishable from odors healthy mice housed with healthy conspecifics and more closely resembled odor profiles of sick animals. Thus, exposure to sick individuals, even in the absence of transmission, could impact how animals are perceived and behave. Plasticity in production of and responses to sickness signals could have complex effects on disease dynamics by modifying social networks.

**Talk: Disease detection in human olfaction**

M.J. Olsson  
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Contagious diseases have been a fatal threat to people throughout evolution. Recent research suggests that behavioral avoidance of sick individuals is the first, and probably most
cost effective, line of defense against infection (Schaller, 2011). Indeed, statistical models show substantial disease containing effects from small adaptations in patterns of interindividual contact (Cole, 2006). In addition, behavior can be seen as a vital part complementing and even regulating the classical immune system. This behavioral defense and its consequences are poorly understood and surprisingly few studies exist. We have in a series of studies examined the olfactory cues by which we detect disease; the neural mechanisms underlying disease avoidance; and how olfactory disease detection prepares the body for an attack together with classic immunity. In doing this we have utilized an experimental sickness model involving the induction of innate inflammation with an endotoxin (lipopolysaccharide) injection in otherwise healthy participants. Results show that after a few hours of systemic inflammation we smell more aversive from the skin, the urine changes in character and show heightened concentrations of Pyrrole. Results also show that disgusting odors (believed to work as olfactory disease cues) increase levels of an inflammatory marker (tumor necrosis factor alpha) in the saliva. Altogether these results support the importance of olfaction in behavioral immunity.

**Talk: The smell of weakness: costs and consequences of injury for social odor cues in ring-tailed lemurs**

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Animal odorants contain information about stable and transient aspects of the signaler’s condition salient to conspecifics. Thus, condition-dependent odors can mediate social interaction, group dynamics, and successful reproduction in vertebrates, including primates. Scent signals are presumably energetically expensive to maintain, but evidence of production costs derives mainly from experiments on the effects of parasitism and infection in laboratory rodents. We used analytical chemistry and bioassays to examine the effects of conspecific-induced injury on olfactory cues in an aggressive, socially complex strepsirrhine, the ring-tailed lemur (Lemur catta). Some injured animals received antibiotics, so we tested both the (1) ‘costly signal’ and (2) fermentation hypotheses – the latter positing that symbiotic bacteria inhabiting scent glands synthesize volatiles used by the host. We analyzed 111 genital gland secretions collected before, during, and after 33 injuries involving 12 males and 10 females. Normal, ‘healthy’ glandular secretions were significantly dampened during injury, regardless of antibiotic treatment; secretion composition was differentially altered by injury versus medication. In bioassays, male lemurs directed significantly more competitive counter marking’ behavior towards odors from injured than healthy conspecifics, potentially relevant to same-sex competition and dominance interactions. We suggest (1) a cost to odor production borne by the signaler and (2) a role for fermentative bacteria in signal
production. To our knowledge, this is the first evidence that mammalian olfactory signals are perturbed by injury, independent of infection or disease.

**Lunch Break**

**Thematic Session VI: Semio-chemical Methodologies and Analyses** (Chair CD Buesching)

**Talk: Complexity of Scent Marking in Rats: Behavioural Characterisation**

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Many rodents use chemical signals to communicate, depositing important information in the environment in the form of scent marks. These scents play a fundamental role in advertising information to conspecifics such as identity, sex, age, dominance, reproductive receptiveness and health status. Norway rats (Rattus norvegicus) are a highly sociable rodent species, often living in large groups competing for resources, territory and mating opportunities, and thus rely heavily on chemical signals to maintain social structures. Rats scent mark by lowering their anogenital region to contact objects in their environment, often leaving visible deposits of urine and/or sebaceous secretions. However, relatively little is known about the composition of scent marks. Molecular analyses of male and female marks demonstrate that deposits are not simply derived from urine. The preputial and prostate glands in males, and the clitoral glands in females, contribute to the protein composition.

We examined scent marking behaviour in response to presence or absence of stimuli, as well as the behavioural responses to naturally deposited scent marks and components from different sources, to determine their potential role in intraspecific communication. We show that rats are highly sensitive to the odour cues from scent marks and components from different glands, and each elicit sex-specific responses. Male rats may also ‘tune’ the contents of their scent marks according to context. These findings provide novel insights into the complexity of rat scents and the role of different scent components on the natural behavioural responses of males and females.

**Talk: PTR-TOF-MS as a tool for studying vertebrate chemical ecology: a case study on the use in kairomone determination in a predator-prey context**

C. Van Moorleghem\(^1\), M. Portillo-Estrada\(^2\), Y. Lambrechts\(^1\), I.A. Janssens\(^2\) & R. Van Damme\(^1\)

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In the wake of improvements in chemo-analytical methods, the field of chemical ecology is rapidly advancing. However, particularly in vertebrates, the true nature of informative compounds in specific contexts remains largely unexplored. Here, we validate the implementation of a recently developed tool used to determine Volatile Organic Compounds (VOCs), to improve the identification of semiochemicals in a wide variety of animal interactions. The existing technique is called Proton Transfer Reaction Time-of-Flight Mass Spectrometry (PTR-TOF-MS). The accompanying procedure has the advantage of not requiring sample preparation which, together with the device’s high accuracy and low detection limit, enables us to measure even the most volatile molecules that often are lost with other techniques. In this study, we apply this chemo-analytical method for determining candidate kairomones used by viviparous lizards (Zootoca vivipara) to detect and avoid saurophagous snakes, such as the viper Vipera berus. Firstly, behavioural observations indicated that lizards can use airborne chemicals for predatory risk assessment. Subsequently, with PTR-TOF-MS, a VOC profile was made of Vipera berus scent and several chemical compounds were put forward as candidate information carriers on predatory risk. Furthermore, the effect of odour fade-out on lizard behaviour and chemical composition was investigated. Our results show that PTR-TOF-MS could complement more widely used methods in resolving important unanswered questions in vertebrate chemical ecology.

Talk: Non-invasive sampling of body odour to investigate the information content of meerkat scent

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\textsuperscript{6}German Center for Integrative Biodiversity Research (iDiv), Leipzig, Germany

The olfactory cues present in an animal’s body odour mediate a wide range of mammalian social interactions by encoding information about individual attributes such as identity, sex, age, or group membership. The chemical composition of these cues provides important indications of the ecological and evolutionary processes shaping olfactory communication. However, while olfactory communication is frequently addressed in behavioural studies, the compounds involved in chemical interactions between individuals have only been sparsely investigated in most mammalian taxa and contexts. We adjusted and evaluated the use of thermal desorption (TD) tubes, commonly used in plant metabolomic and environmental studies, for non-invasive sampling of meerkat (Suricata suricatta) body odour. Adjustment of sampling parameters (e.g. distance, volume, flow rate, interruption of sampling) to increase feasibility for a non-invasive application yielded samples of adequate quality. TD tubes
captured a wide range of volatile and semi-volatile organic compounds including those likely originating from the target animals. The composition of the body odour profiles differed between groups, age classes and body regions, but not between the sexes. These results corroborate findings of behavioural studies on meerkat olfactory communication and on differences in bacterial communities in meerkat scent secretions. Our study indicates that TD tubes are suitable for non-invasive sampling of body odour, making them an attractive tool for addressing a wide range of questions in the lab and the field.

Talk: The male effect: finding a way through the jungle of chemical cues

C. Le Danvic 1, M. Dillenbourg 1, M. Keller 2, L. Schibler 3, P. Nagnan-Le Meillour 4

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In ungulates, the introduction of a sexually active male in an anoestrus female herd induces a surge of LH leading to ovulation. This process conventionally used by breeders in their flock, known as "male effect", allows the control reproduction and appears to be also an interesting alternative to the use of hormone. Male effect depends predominantly on olfactory stimuli generated by the male (Okamura & Mori, 2005; Cohen-Tanoudji et al., 1994). If some potentially involved molecules have previously been identified (Cohen-Tanoudji et al., 1994 ; Murata et al., 2014), the complete signal (those inducing ovulation) remains to be clearly characterized. Using an original approach based on the comparison of olfactory profiles of males during non-reproductive and reproductive periods, we undertook the complete “male effect” pheromones characterization in French breeds. Various biological samples (urine, wool, head secretions) that could be responsible for male effect were collected and chemical profiles were determined by GC/MS analysis after SPME or solvent extractions. Specific ram and bulk olfactory profiles have been observed during the period of maximal sexual activity with 1/ a global increase in intensity profiles and 2/ the appearance of numerous molecules. In total, more than 60 (on 147) molecules season-specific (Fold-change>10) were characterized (ethyl esters, ketones, ...). We are now focusing our interest on those molecules to validate their biological activity on female. Discussions are currently conducted with our partners (INRA, CNRS) to define the best modalities to assess their biological activity. The long-term objective is to develop a difuser allowing to mimic/potentiate the male effect in breeding farms.

Talk: Comparison of different analytical methods for pre-processing gas chromatographic data in semio-chemical research

C.D. Buesching 1, H.V. Tinnesand 2, C.T. Mueller 3 & M.J. Noonan 4

1 Wildlife Conservation Research Unit, University of Oxford, Zoology Department, The Recanati-Kaplan Centre, Tubney House, Abingdon Road, Tubney, Abingdon, OX13 5QL, UK;
Recent advances in sensitivity and cost-effectiveness of GC-MS techniques have resulted in a drastic increase of semio-chemistry research. Problematically, however, GC-MS data do not carry absolute, but rather relative information, and thus even small differences in sample volume, concentration, and/or lab procedures, in combination with background noise, can influence the perceived abundance of compounds. Because of this, pre-processing must be carried out before data can be compared statistically. Currently, researchers use a wide range of pre-processing techniques, often unaware of some of their intrinsic properties and limitations. Here, we use a combination of 20,000 simulated profiles, and empirical GC-MS data from 15 samples of badger (*Meles meles*) subcaudal gland secretion, to test the performance of the most commonly used normalisation methods (Total Sum Normalisation ‘TSN’; Probabilistic Quotient Normalisation ‘PQN’; Internal Standard Normalisation ‘ISN’; External Standard Normalisation ‘ESN’; and a compositional data approach ‘CODA’).

We demonstrate that each method imparts unique properties to the data, and any ecological inference is thus likely to be influenced by the normalisation technique employed. Calibrating against an external or internal standard provided good results when differences were pronounced, but were less reliable for more subtly differentiated groups. The reliability of TSN and CODA approaches, however, were highly dependent on the structure of the data, and prone to generating erroneous conclusions. The low false positive rate of the PQN approach made it the most generally applicable normalisation method, whereas the suggestion of utilising a blanket approach of first applying multiple different normalisation methods and then identifying results that are consistent across each is unlikely to be effective for all datasets.

**Talk: Sampling and analysis of VOCs & SVOCs – What are the best tools for the job?**

Massimo Santoro, Ilaria Ferrante, Hannah Calder

Markes International Ltd., Gwaun Elai Medi-Science Campus, Llantrisant, UK

Thermal desorption (TD), in conjunction with gas chromatography (GC), is a powerful and versatile technique that enables the detection and quantitation of trace organic chemicals from ambient air, workplace environments as well as those released from materials. More recently this well used technique has been adopted by the fields of chemical ecology and biological monitoring and offers fascinating insights into the complex interactions involving plants, microorganisms and animals as well as the detection of disease markers and odour analysis of breath. This presentation will focus on the various sampling techniques that facilitate the use for this technique across a wide range of applications and discuss the
benefits of the analytical system for detection and quantitation of Volatile and Semi-volatile organic compounds.

**PM Coffee Break**

**Open session 4**

**Talk: Temporal chemical profiling of urine volatile compounds of domestic cats and their olfactory discrimination abilities**

C. Suzuki, M. Maita, T. Miyazaki, T. Yamashita & M. Miyazaki

Territorial animals deposit their urine and feces on their territories. These excretions contain thousands of metabolites some of which may be used as scent signals for species, sex, age, and individual recognitions. Previous studies have suggested that compounds identified species-, sex-, and age-specifically and varied among individuals are important for scent communication. In these studies, fresh excretions were used for their chemical profiling and behavior bioassays. However animals usually encounter excretions after aging periods of several hours and days in their territories. Therefore, it is necessary to analyze not only fresh excretions but also aging excretions in animals. In the present study, we examined temporal changes of chemical profiles of urinary volatile compounds of domestic cats, and their olfactory discrimination abilities toward these volatiles. Cottons were infiltrated with male cat urine, and then the headspace gas emitted from the cottons before and after air-drying were analyzed in two-dimensional gas chromatography (GC×GC)-mass spectrometry (MS). GC×GC-MS detected over two thousands of volatiles in both headspace gas emitted from fresh and aging urine. Chemical profiles of the headspace gas differed markedly between fresh and aging urine. There was a tendency that contents of compounds with low-boiling point decreased in aging urine, while compounds with middle-boiling point increased. In behavioral assays, cats which habituated to fresh urine showed dishabituation to aging urine. These results indicate aging urine emitted more compounds with middle-boiling point than fresh urine, and cats can distinguish differences of chemical profiles between fresh and aging urine by olfaction.

**Talk: Identification of key compounds for the individual recognition in the domestic cats**

M. Maita¹, C. Suzuki¹, J. Caspers², T. Yamashita¹, S. Schulz² & M. Miyazaki¹

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Domestic cats spray their urine onto a vertical surface for scent marking. They sniff urine deposited by other individuals of intraspecies carefully, and then exhibit the flehmen response; the cats raise their head and hold their mouth partially open for a few seconds. Our previous studies demonstrated that volatile felinine derivatives were strong candidates of scent signals for species, sex, and age information in cats. However, little is known which
compounds are involved in individual recognition in cats. The aim of this study was to identify key compounds for individual recognition in cats. It is known that the flehmen response is observed in males that sniff estrous female urine in cats. In addition, we found that cats exhibited the flehmen response to other cat urine of same sex, but not own urine. We purified key compounds from cat urine, using sniffing time and the flehmen response as indicators of individual recognition. Our analyses indicated that cats sniffed urinary compounds containing of a functional group of lipids with considerable interests and showed the flehmen response. In GC-MS analyses, urinary contents of these compounds were varied among individuals. Habituation-dishabituation tests showed that cats distinguished the difference of urinary contents. Furthermore, chemical profiles of these compounds were conserved between fresh and aging urine in each cat. These results indicate that the lipids are key compounds for individual recognition in cats.

Friday 01 September 2017

**Plenary: Scent wars: unravelling the complexity of competitive scent signalling**

J.L. Hurst

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Competitive scent marking is the most overt form of scent signalling seen in mammals, involving a battle fought between individuals or social groups that is played out at both behavioural and molecular levels. Competition, particularly in the context of reproductive opportunity, often leads to a dramatic increase in investment in specific molecular components of scents as well as in behavioural deployment. However, an individual’s ability to produce and deploy these signals is regulated through competitive aggression, leading to honest signalling of competitive ability. Studies in rodents – most particularly in house mice – have identified multiple components that contribute to these dynamic signals, potentially providing extensive information about the owner to competitors and to potential mates. This includes several androgen-dependent volatile pheromones together with MUPs (major urinary proteins), which are a multigene family of involatile urinary lipocalin proteins that bind and control the release of volatiles from deposited scent marks. One male-specific MUP, darcin, plays a key role in inducing female mice to rapidly learn attraction to the odour and location of ‘winning’ males that countermark their competitors’ scents. Most investment, though, is in another set of MUPs that provide a distinctive and surprisingly stable signature of owner identity at several levels. This MUP signature further determines the volatile signature that females learn from male scent marks. Increasing the rate of scent deposition and amount of MUP investment thus increases the strength of an individual’s scent in the face of competing signals, providing a combined signal of identity and competitive status.
Thematic Session VII: Olfactory advertisement (Chair Jane Hurst)

Talk: Urinary volatiles and bioindicators related to reproductive status in giant panda (*Ailuropoda melanoleuca*)

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In ex-situ panda breeding, urinary steroids serve as estrous indicators and to time AI. Following a period of a delayed implantation, female pandas generally experience pseudopregnancy. However, if a true pregnancy occurs, a placenta is formed that may affect the composition of urinary compounds. We aimed to: 1) analyse urinary volatiles that adsorb on 1 cm pieces of polydimethyl-siloxane (PDMS); 2) detect estrous related substances; and 3) find compounds that might be applicable to differentiate true pregnancy from pseudopregnancy. An Agilent GCMS was used for the analyses of PDMS adsorbed substances using 1 ml of urine. Compound profiling was carried out using the freely available XCMS software at http://metlin.scripps.edu. Analyses of PDMS adsorbed urinary volatiles confirmed earlier results of an abrupt increase in fatty acids from basal to peak levels 6 days prior to ovulation. However, fatty acid composition appears to vary between consecutive years and between individuals. In addition, substances such as D-limonene and 2-ethyl-1,4-dimethyl-benzene were detected peaking around estrous. Comparison of urinary compounds between pregnancies and pseudopregnancies from one panda during the secondary phase (high levels of progestagens following assumed implantation) revealed several significantly elevated but unidentified substances during true pregnancy. Moreover, one of those substances was also significantly elevated during the primary phase (low levels of progestagens after conception). However, no substance was found exclusively during true pregnancy. As results were obtained from only two pandas, more samples are needed to confirm if a pregnancy specific substance exists in this species.

Talk: Females prefer cooperative males based on cues different from cooperative behaviours: evidence from the Mound-building mouse, *Mus spicilegus*

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Theoretical and empirical studies in humans suggest that cooperative behaviors may act as signals during mate choice. However, cooperation is not always observable by potential partners before mate choice. To address whether cooperative phenotypes are preferred based on cues different from cooperative behaviors per se, we designed an experimental paradigm using wild-born Mound-building mouse (*Mus spicilegus*) a species with biparental care. In this species, females cannot observe male cooperative behaviors: mate choice occurs in spring, whereas mounds are cooperatively built in fall. We first assessed variation
in mound building investment and identified high and low cooperative males. Second, we presented these males to females during two-way choice tests. Our results indicate that females were more attracted to high over low cooperative males, even when they did not observe them build. This finding suggests that female mate choice is influenced by cues of cooperativeness different than cooperative behaviors per se. We speculate that some chemosensory cues of mate quality or cooperativeness could be involved in this detection.

**Talk: Sex-steroid excretion in urine as olfactory advertisement in European badgers (Meles meles) in relation to circulating plasma titres.**

N.A. Sugianto1, H.V.2, D.W. Macdonald1 & C.D. Buesching3

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2Faculty of Arts and Sciences, Department of Environmental and Health Studies, Telemark University College, N-3800 Bø i Telemark, Norway

Circulating sex-steroid levels are a reliable indicator of an individual’s reproductive status. After metabolization, sex-steroids are removed from the blood stream through excretion in urine via the renal system, or in faeces via the liver. Therefore, these metabolic waste products may serve as honest and reliable olfactory reproductive advertisement signals to conspecifics. The European badger (Meles meles) is a highly macro-osmatic mustelid and uses glandular secretions as well as urine and faeces for intra-specific communication. Here, we analysed 127 urine samples collected from 58 male and 69 female badgers in Wytham Woods (Oxfordshire-UK) during the post-weaning period for females and main mating season in spring (May/June), during the reported secondary mating peak in summer (August/ September), and during reproductive quiescence in autumn (November), and used GC-MS to determine how urinary sex-steroid metabolites differ between individuals of different reproductive states. GC-MS profiles varied with circulating plasma levels of oestradiol (measured with EIA using oestradiol-17-HS-BSA-antiserum), oestrone (EIA using polyclonal-EC-R522-antiserum) and testosterone (EIA using anti-testosterone-R156/7). As badgers are reported to both, sniff and lick urine from conspecifics, we infer that they likely use volatile as well as non-volatile components to decipher urinary information content.

**Talk: Scent-marking as Honest Fitness Advertisement in European Badgers (Meles meles)**


Wildlife Conservation Research Unit, Department of Zoology, University of Oxford, Recanati-Kaplan Centre, Abingdon Road, Tubney House, Tubney, Oxfordshire OX13 5QL, UK.

Scent-marking is considered costly not only due to the physical activity associated with depositing scent marks with adequate frequency and of adequate strength, but also due to the physiological production of the secretions themselves. Factors related to honest fitness advertisement – particularly individual variation in secretion composition – due to intrinsic (e.g. age, sex, genetics) and extrinsic (e.g. resource availability, population density, climate) factors can impact the perceived attractiveness of scent-marks. Therefore, scent marks have been suggested to function as honest
fitness advertisement signals. However, little research has investigated individual energetic costs toward secretion production. European badgers (*Meles meles*) are highly macrosmatic mustelids. Like all carnivores, they possess paired anal glands, but have evolved an additional unique subcaudal gland. Subcaudal gland secretion is rich in long-chained carboxylic acids and encodes for individuality and group-membership, and a wealth of fitness-related parameters, such as sex, age, reproductive and somatic condition. Here we measured the size of the subcaudal gland opening as a proxy for scent mark size, and analysed the energy content of subcaudal gland secretions, using bomb calorimeter analyses. We relate opening size and calorimetric differences to sex, age and body condition, and discuss the suitability of subcaudal gland secretions as honest (i.e. unfakable) fitness advertisement signals.

**AM Coffee Break**

**Poster Talks 4: Advertisement**

**POSTER 14: Changes in composition of urine during various periods of ovarian cycle in female of domestic dogs (*Canis familiaris*)**

M. Dzięcioł, A. Szumny, W. Niżański, T. Jezierski, M. Woszczyło & B. Pieczewska

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Since many reports undermines the meaning of methyl paraben as a sex pheromones in dogs, the aim of this study was to evaluate changes in composition of the urine in female dog (*Canis familiaris*) during the different phases of ovarian cycle. More than hundred of urine samples collected from the various females during the particular phases of ovarian cycle were evaluated with the HS-SPME/GC-MS technique. The phase of the cycle was confirmed by evaluation of clinical symptoms as well as by vaginal cytology and hormones level measurement. The results showed significant differences between samples collected during particular phases of ovarian cycle. About thirty compounds were identified in urine samples. Several differences in chemical profile of volatile compounds as affected by ovarian cycle were noticed. Unequivocally, the level of carbonyl aromatic compounds, such as acetophenone (hypnone) and benzaldehyde increased in the oestrus cycle. It was accompanied by rising the methylketones, e.g. butanone, 2-pentanone, 3-hexanone. Simultaneously the sulfide compounds: 1-methylotio propane; 1-methylotio butane, 1-methylotipentae or dimethyl trisulphide significantly satistically decreased in period of oestrus and rised in dioestrus. This observation suggest possible dual character of mechanism of interaction between males and females during mating period including both luring but maybe also repellant signal existence. Contrary to Goodwin et al. (1979) observation, no parabens were found in examined samples. The further study including the
behavioral tests are indicated to verify semiochemical activities of identified substances and genuineness of thesis about luring/repeling mechanism involved in mating behavior in dogs.

POSTER 15: Scent Communication in Bank Voles: Does male investment influence female response?

H. Coombes¹, Stockley¹, R.J. Beynon² & J.L. Hurst¹

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Olfactory communication plays a key role in many mammalian social interactions and many animals invest heavily in depositing scent signals. However, the molecular composition of scent marks, why animal invest in scent signals and behavioural response of conspecifics to individual scent cues is poorly understood in most non-model species. Scent marking is important in vole social behaviour and scent signals are a key source of information about conspecifics. In this study, we examine scent marking behaviour in bank voles (Myodes glareolus). We investigate whether male bank voles invest differentially in competitive scent marking, both in terms of the number of marks deposited and the protein composition of these scent marks. We also explore whether individual male scent marks elicit different behavioural responses from female voles, and whether females show a preference between males according to their investment in scent marking. Understanding the context in which scent signals are produced, their molecular composition and the behavioural responses they stimulate, each provide important insight required to understand how chemical communication influences social behaviour.

POSTER 16: Exploring VOCs from harbour porpoise excreta

M. Gabirot¹, B. Bouchard¹, A. Célérier¹, L. IJsseldijk² & S. Campagna¹

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Cetaceans are descendants of land-living mammals which returned to water for a fully aquatic life. This singular evolutionary history is accompanied by a profound reorganization of the chemosensory system (olfaction and gustation). In toothed whales particularly, this is illustrated by the loss of anatomical structures as olfactory bulbs and nerve. However several reports based on behavioural experiments with bottlenose dolphins demonstrate the presence of an effective chemical perception in feeding as well as social context. Thus if chemicals are involved in social interactions in cetaceans, informative compounds should be present in the excreta of these animals. For this reason, we have launched the analysis of the volatile organic compounds (VOCs) released from the urine and faeces of dead harbour porpoise (Phocoena phocoena). We have developed for that purpose a novel and robust methodology using solid phase microextraction (SPME). The comparison of VOCs found among the excreta from individuals of different ages, sexes and health status with those present in the excreta from terrestrial mammals will allow us to detect the presence of social semiochemicals in marine mammals. This innovative work could also have major
implications in monitoring the health status of animals based on the possible presence of disease markers in the excreta.

**POSTER 17: Putative pheromone of snow leopard, Panthera uncia (Schreber, 1775)**
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Chemical characterization of urine of snow leopard was carried out to investigate its pheromonal significance. Panthera uncia syn. Uncia uncia (Schreber, 1775), a crepuscular and solitary endangered felid, is the native of North Eastern Himalayan mountain range of Indian subcontinent. Observations on urine were conducted on semi-captive animals of Padmaja Naidu Himalayan Zoological Park, West Bengal, India (27°03'30.1"N 88°15'14.4"E). Solid phase microextraction (SPME) of headspace volatiles of the urine from snow leopard were examined by gas chromatography–mass spectrometry. About 32 low carbon compounds with various functional groups like alcohols, aldehydes, ketones, phenol, amines, some cyclic hydroxy compounds and a sulphur containing compound have been identified. The volatilome of urine of snow leopard revealed the presence of 6-methyl-5-hepten-2-one, the principal component of lemon grass oil and the major pheromonal component of red fox, Vulpes vulpes. We detected this specific aroma by smelling, conducted on about twenty volunteers when sprayed over filter paper. Various branched and unbranched saturated and unsaturated fatty acids from C12–C18 were identified from the lipid, extracted from urine. However, significant variation in composition of volatilome and lipidic fraction of urine of snow leopard from tiger, leopard, lion and African cheetah might be indicative for their different strategy and nature of territorial marking. It might be relevant to their different origin in different ecological environment.
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