1 Phenolics: old-fashioned chemistry in extant julid millipedes (Julida, Diplopoda)?

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Diplopods are well known to produce repellent and noxious fluids from serial exocrine glands. Depending on the taxonomic group, they show considerable diversity in the composition of their secretions. The Juliformia, for instance, are known for their quinone-rich exudates (”quinine millipedes”). However, additional reports of non quinonic compounds have been reported. One class of such “aberrant” pungent smelling compounds is the phenols, which actually characterize the defensive secretions of the close juliformian outgroup Callipodida. Within the Juliformia phenols have only been reported for two julid species. We here conducted a chemical screening with focus on the occurrence of phenols in julid millipedes, to further contribute to the phenomenon of phenolic secretions in juliformians. Defensive secretions of 42 species belonging to 17 genera and 6 tribes of Julidae were analyzed by gas chromatography – mass spectrometry. Phenol-rich secretions were found in 6 species from 3 different tribes. In detail, pcresol was found to be a major compound in the cylindroiulines Styrioiulus pelidnus and S. styricus and an undetermined Cylindroiulus species, in the brachyiulines Brachyiulus lusitanus and Megaphyllum fagorum, as well as in a yet undescribed Typhloiulus species (Leptoiulini). In all 6 species p-cresol was accompanied by small amounts of phenol. Except for M. fagorum, which produce exclusively phenolic-compounds, phenols were also accompanied by different quinones. Biosynthetically, benzoquinones and phenols are supposed to share a common pathway, leading from aromatic precursors to phenols and finally to benzoquinones. With respect to phenolic secretions in juliformian outgroups (Callipodida, Polydesmida, Stemmiulida), we hypothesize that phenols represent remnants of an ancestral chemical equipment which having been replaced by benzoquinonic compounds in most juliformian taxa.

2 Novel set-up for low disturbance sampling of volatile and non-volatile compounds from plant roots.

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Most studies on rhizosphere chemicals are carried out in substrate-free set-ups or in artificial substrates using sampling methods that require an air flow and may thus cause disturbance to the rhizosphere. Our study aimed to develop a simplified and inexpensive system that allows analysis of rhizosphere chemicals at experimentally less disturbed conditions. We designed a mesocosm in which volatile rhizosphere chemicals were sampled passively (by diffusion) without air- and water flow on polydimethylsiloxane-(PDMS) tubes. Dandelion (Taraxacum sect. ruderalia) was used as model plant; roots were left undamaged. Fifteen volatiles were retrieved from the sorptive material by thermal desorption for analysis by gas chromatography/mass spectrometry (GC/MS). Furthermore, three sugars were collected from the rhizosphere substrate by aqueous extraction and derivatized prior to GC/MS analysis. In order to study how the quantity of detected rhizosphere compounds depends on the type of soil or substrate, we determined the matrix-dependent recovery of synthetic rhizosphere chemicals. Furthermore, we compared sorption of volatiles on PDMS tubes with and without direct contact to the substrate. The results show that the newly designed mesocosm is suitable for low-invasive extraction of volatile and non-volatile compounds from rhizospheres. We further highlight how strongly the type of substrate and contact of PDMS tubes to the substrate affect the detectability of compounds from rhizospheres.

3 Chemical communication in spiders: SEM-analysis of the distribution of chemosensory sensilla on male *Argiope bruennichi* (Araneidae)

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Olfaction is considered the prime channel of communication in spiders, irrespective of whether they are cursorial or web-building. However, we know surprisingly little about which chemical signals or cues are involved in olfaction in spiders and only a single sex pheromone responsible for long distance mate attraction is known to date. Even less is known about the receptors involved: s-shaped and blunt tipped sensilla with a subterminal pore are considered to be responsible for olfaction in spiders. So far, electrophysiological analysis demonstrated the perception of contact pheromones with these sensilla and it is generally assumed that the same structures are responsible for long distance olfaction. We investigated the structure and distribution of the supposed chemosensory sensilla along with other sensory organs on all walking legs and pedipalps of Argiope bruennichi males by means of Scanning Electron Microscopy. We show that A. bruennichi males possess tip-pore sensilla on all legs and the palps. On the tarsi and metatarsi of the walking legs, sensilla density is high whereas on tibiae and patellae sensilla are rare. On coxae and trochanters no sensilla were found. On the male pedipalp, chemosensitive sensilla occur exclusively on the cymbium, a spoon shaped structure at the end of the pedipalp. Our results will help to perform targeted electrophysiological studies with the synthetic sex pheromone. We will test if the tip-pored sensilla are able to perceive the volatile sex pheromone and if sensilla of specific regions show higher sensitivity.

4 Chemical signaling in Poeciliid fishes: Analysis of released substances and their relevance during mate choice

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The olfactory system of fish plays, for example, an important role in predator recognition, kin or individual recognition, species recognition, as well as in the discrimination between sexes. During intersexual interactions, fishes release pheromones that attract individuals of the opposite sex. Moreover, in species exhibiting shoaling behavior, females are attracted by olfactory cues released by conspecific females. Also in livebearing Poeciliid fish, substances that are released into the water seem to act as important cues during mate choice. Sailfin molly (*Poecilia latipinna*) males, for instance, show differently strong sexual behavior towards females of different fertility status and mating status. Hence, they seem to be able to assess these factors chemically. So far, the whole spectrum of chemical substances responsible for an olfactory-based preference of one individual over another has not been identified in Poeciliid fish. Ramsey et al. (2011) found that endogenous 17-ß-estradiol levels vary across the reproductive cycle of females in the swordtail X. nigrensis and that the amount of 17-ß-estradiol that is released into the water correlates with the endogenous level. Beyond this finding, it is yet unclear how the composition and/or amount of chemical substances released by females differ between individuals of different receptive stages and of different mating status. Moreover, it is unknown whether and how chemical profiles (i.e. the mixture of chemical substances released into the water) of males vary with sexual motivation. I plan to conduct quantitative and qualitative chemical analyses to identify the relevant chemical substances that male and female sailfin and Atlantic mollies (*P. latipinna* and *P. mexicana*) release into the water. These substances might represent important chemical cues that supply other individuals with information about species, sex and mating/fertility status. In our pilot study we collected water samples from glass tanks (each holding 5 l of water) in that we placed one sailfin molly (male or female) for six hours. These water samples were analyzed using electrospray ionisiation high-resolution mass spectrometry, ESI-MS. Three fish-related compounds and corresponding glucosiduronic derivates, respectively, could be readily identified to be present in the tank water: bile acid deoxycholic acid (DCA), and the steroids 17.21-dihydrox-3-3oxopregn-4-en and 3-hydroxy-5-androstane-11.17-dione. Identification was achieved by using an Orbitrap high-resolution mass spectrometer (R = 100.000) with high mass accuracy (< 3 ppm). All three substances were previously reported in other fish species and were attributed to act as pheromones. The here detected substances were found in both, the male and the female sample. Several other chemical substances were detected, but identification was hindered by low abundances in the mass spectrum and potential interferences. For future analyses, the analytical protocol needs to be optimized and refined. With my planned analyses, I hope to be able to deliver the chemical background for behavioral observations in choice experiments.

5 Analysis of volatile compounds from body odor samples with GC-MS after thermodesorption from sorbent tubes

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Body odor samples of common marmosets were collected by adsorption to the porous polymer Tenax TA. After thermodesorption (TD) with subsequent gas chromatography – mass spectrometry (GC-MS) analyses of these samples, volatile organic compounds (VOCs) were identified and assessed for their chemical classes, boiling points, and retention times. From those, 60 compounds were selected resembling the representative pattern of these samples covering a wide elution range (bp. 20-256 °C) and considering at least ten chemical classes. A model mixture was prepared at equimolar concentrations of the neat standards of these 60 compounds and introduced by liquid spiking into sorbent tubes of two porous polymers (Tenax TA and XAD-2). VOCs were analyzed by TD-GC-MS to evaluate recovery, sensitivity, and reproducibility. Good chromatographic separation was obtained for most of the standards eluting at later retention times, while insufficient chromatographic performance was observed for the more volatile compounds appearing as broader peaks, and overlapping eventually due to insufficient cooling of the trap in the thermodesorber (Tmin 30 °C). The high noise level observed in the chromatograms was related to sorbent degradation but could be decreased without relevant changes in recovery and sensitivity after reducing the desorption temperature of the sample tubes. The assessment of the peak areas by selective mass traces revealed high sensitivity levels (0.1-10 ng) for most of the compounds in the standard mixture. Linearity ($R2>0.99$) was determined with at least five calibration levels reaching concentration ranges between 0.1 µM and 10.0 mM ± <10 % RSD. Analysis of the model mixture finally confirmed the identification of 29 volatile compounds in odor samples collected by two-bed sorbent tubes from genital secretions of female marmosets during menstrual cycle stages (n=4).

6 A novel non-terpene from the exudates of Aphids

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After mechanical stimulation, aphids often release sticky secretions from their cornicles to defend themselves against predators. In many species cornicle secretions often contain volatile constituents serving as alarm pheromones. A widespread volatile among aphid species is the sesquiterpene (E)-ß-farnesene (EFB), but other sesqui- and monoterpene compounds have been additionally described. Nevertheless, chemical communication in aphid colonies still deserves further investigations because many aphid species are still chemically unexplored and complete chemical profiles of most species are missing. In this study we used gas chromatography-mass spectrometry to study volatiles of over 100 aphid colonies that were collected in different natural habitats in Austria, Hungary and Italy. In several samples we found tetradecyl actetate that is known to act as a sex pheromone in some moth species, but has not been described in aphids so far. With the exception of EBF this molecule was the second most common substance in all probes. It was either present as the only component or occurred together with EBF and smaller monoterpenes. Surprisingly, it often exceeded the relative abundance of all other components. Future studies will shed light on the behavioral relevance of this molecule.

7 Chemo-visual Flower Preference of Alternative Pollinators incorporating TDU-GC/MS-EAD

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Plants exploit a range of strategies to attract pollinators including scent, colour, morphology, humidity, nectar etc. Floral scent acts as a long range signal for pollinators. Hoverflies are cosmopolitan across the globe and play a major role as alternative pollinators to bees and bumblebees. We have observed similar populations of hoverflies in climates ranging from subarctic Sweden, temperate Germany, alpine Himalayas, and tropical south India. We thus ask the question: What are the chemical and visual cues that allow these pollinators to locate flowers in such diverse climates? Combining field observation, image analysis, spectrophotometry, abiotic measurements and chemical analysis, we have investigated the chemo-visual cues used by hoverflies across various climates and geographies. Solid phase extraction using polydimethylsiloxane (PDMS) was used to collect floral volatiles from different regions at each visited/non-visited flower. These volatiles were desorbed by thermal desorption and analysed using coupled gas chromatography/-mass spectrometry. Identified floral volatiles were further corroborated for the perception of hoverflies by electroantennographic detection. Detailed methodology of extraction, analysis and identification of floral volatiles with outcomes will be discussed in the presentation.

8 Extreme variation in floral scent and form in the sexually deceptive genus *Ophrys*: consequences for reproductive isolation and speciation

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Floral traits have been shown to often reflect adaptations to particular pollinators or pollinator groups. Variation of floral traits may therefore arise through pollinator-mediated selection. Depending on the specificity of the traits in ensuring pollinator attraction other selective and neutral factors may also become important. In deceptive pollination systems the mechanism driving floral trait variation and its consequences for reproductive isolation and speciation are still insufficiently understood. The sexually deceptive orchid genus *Ophrys* is one of the most species rich plant genera in the Mediterranean region, its diversity being mainly attributed to its highly specialized pollination system and multiple pollinator shifts. Because the genus is notorious for high floral variability, the effectivity of pollinators in shaping floral isolation has been questioned. Our study therefore aimed to assess the relative role of pollinators and other factors in shaping the amount and patterns of inter- and intraspecific variation in floral scent, shape and size. We thereby focused on three *Ophrys* species occurring on several isolated islands within the mosaic landscape of the Aegean Sea. Our results show that species specific divergence in floral traits between pollinator-delimited species is maintained despite high amounts of variation. In fact pollinators have been found to be the main drivers of variation in floral scent and size even in the face of strong isolation and small effective population sizes. In turn floral shape appears to be more strongly influenced by genetic drift. The high amount of variation in floral traits appears to be linked to the degree of specificity and to be the outcome of an interaction between pollinators, geographic isolation and drift. In fact our study is the first to explicitly underline the importance of the geographic mosaic build by the multitude of small islands, characteristic for the Aegean Sea, for the diversification of the genus *Ophrys*.