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Grant Proposal

Ecology and Evolution of Intraspecific Chemodiversity of Plants

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Abstract

An extraordinarily high intraspecific chemical diversity, i.e. chemodiversity, has been found in several plant species, of which some are of major ecological or economic relevance. Moreover, even within an individual plant there is substantial chemodiversity among tissues and across seasons. This chemodiversity likely has pronounced ecological effects on plant mutualists and antagonists, associated foodwebs and, ultimately, biodiversity. Surprisingly, studies on interactions between plants and their herbivores or pollinators often neglect plant chemistry as a level of diversity and phenotypic variation. The main aim of this Research Unit (RU) is to understand the emergence and maintenance of intraspecific chemodiversity in plants. We address the following central questions:

1) How does plant chemodiversity vary across levels, i.e., within individuals, among individuals within populations, and among populations?

2) What are the ecological consequences of intraspecific plant chemodiversity?

3) How is plant chemodiversity genetically determined and maintained?

By combining field and laboratory studies with metabolomics, transcriptomics, genetic tools, statistical data analysis and modelling, we aim to understand causes and consequences of plant chemodiversity and elucidate its impacts on the interactions of plants with their biotic environment. Furthermore, we want to identify general principles, which hold across different species, and develop meaningful measures to describe the fascinating diversity of defence chemicals in plants. These tasks require integrated scientific collaboration of experts in experimental and theoretical ecology, including chemical and molecular ecology, (bio)chemistry and evolution.

Keywords

Chemodiversity, biodiversity, plant-herbivore interactions, metabolomics, transcriptomics

State of the art and preliminary work

State of the art

Phenotypic differences among individuals within a population provide the raw material for natural selection and, when genetically based, lead to adaptive evolution. The importance of individual phenotypic differences is now recognised in various research fields (Des Roches et al. 2018, Koricheva and Hayes 2018, Müller and Orians 2018), from social sciences to studies in ecology, behaviour and evolution. The level of phenotypic variation within a population is often surprisingly high. For plants, the chemical composition of the different tissues is an important part of the overall phenotype. The huge diversity of specialised (also called secondary) metabolites of plants has been fascinating scientists for decades. While this diversity in metabolites is highly valued as a source for pharmaceuticals, the question why plants produce such an enormous diversity is still wide open. Plant species display varying levels of intraspecific phytochemical diversity (chemodiversity) within and among populations, from very low to extraordinarily high. For example, within tree species such as *Populus nigra* L. (black poplar; Salicaceae) and other *Populus* spp., variation in phenolics and other metabolite classes is found (Boeckler et al. 2013, Moritz et al. 2017). Populations of herbaceous species such as *Solanum dulcamara* (L.) (bittersweet nightshade; Solanaceae) show pronounced differences in their glycoalkaloid profiles between individuals (Calf et al. 2018). In *Tanacetum vulgare* L. (common tansy; Asteraceae) currently more than 30 chemotypes have been described, which are characterised by the dominant leaf and flower terpenoid(s) (Clancy et al. 2016, Keskitalo et al. 2001, Wolf et al. 2011). These three plant species are well studied by members of our Research Unit (RU) and offer highly suitable study systems to investigate the factors contributing to the emergence and maintenance of plant chemodiversity. Like other forms of intraspecific variation, the maintenance of such high intraspecific chemodiversity requires an explanation, because chance effects and most forms of natural

selection usually lead to loss or fixation of some variants (Speed et al. 2012, Wang et al. 2018). Thus, we aim:

1. to characterise intraspecific plant chemodiversity at different levels,
2. to study the ecological consequences of such diversity and
3. to determine, how intraspecific plant chemodiversity is genetically determined and maintained.

The importance of variation in chemical phenotypes of organisms for shaping species interactions has often been neglected, although chemical communication is the most ancient mediator of information exchange among individuals. Specialised metabolites play an important role in the communication between plants and their environment. They serve as defences against herbivores or pathogens but also as attractants for beneficial organisms such as pollinators (Fraenkel 1959, Hartmann 2007). Not only host choice and acceptance but also the developmental performance of herbivores and pollinators is predominantly shaped by plant metabolites (Schoonhoven et al. 2005). The chemical co-evolution model proposes that rapid adaptations by both plants and insect herbivores impose new selection pressures leading to the production of novel metabolites in an evolutionary arms race (Ehrlich and Raven 1964). However, the chemical co-evolution model is questioned, because not every specialised plant metabolite may have a biological effect. For that reason, the “screening hypothesis” (Firn and Jones 2003) predicts that plants need to screen a large number of metabolites to produce at least some effective defences. To gain such chemodiversity, enzymes must be able to accept more than one substrate or to make more than one product (Firn and Jones 2003). These and other evolutionary theories mainly focus on differences between and among different plant species or populations. However, there is experimental evidence that intraspecific variation within populations matters as well. For some plant species it was found that individuals growing in mixed stands of high intraspecific (genetic) diversity perform better compared to plants in monoclonal stands (Bustos-Segura et al. 2017, Crutsinger et al. 2006, Dawson and McCracken 1995, Müller et al. 2018). This phenomenon may be driven by an increased diversity and complexity of the plant-associated community of invertebrates in more genetically and chemically diverse populations, which reduces damage of individual plants and facilitates pollination. The effects of metabolites, and thus also the effects of intraspecific plant chemodiversity, on species interactions can cascade up to higher trophic levels due to plant metabolites being transferred from one level to the next in the food chain (Gols 2008). In addition, intraspecific differences in (herbivore-induced) plant volatiles influence mutualistic interactions with natural enemies of herbivores as well as with pollinators (Clavijo McCormick et al. 2014, Kuppler et al. 2016, Ye et al. 2018).

Differences in metabolite composition do not only occur among but also within individual plants (Hahn and Maron 2016, McCall and Fordyce 2010). This intra-individual chemodiversity includes all plant parts from roots to shoots and reproductive tissues (Jakobs and Müller 2018, Tsunoda et al. 2017). Moreover, such chemodiversity is also measurable at a very small scale such as, e.g., the phloem sap of different leaves (Jakobs et al. 2019, Stolpe et al. 2017b). In addition, metabolites produced by the various plant parts often show pronounced spatio-temporal variation (Ochoa-López et al. 2018). This

chemodiversity particularly large in long-lived trees such as *P. nigra*, where a tremendous diversity in phenolics occurs within individual crowns and over the years (Boeckler et al. 2013, Lämke and Unsicker 2018 and references therein). Our current knowledge about the effects of intraspecific and intra-individual plant chemodiversity on communities of plant-associated organisms and their biodiversity is still incomplete. Therefore, we argue that more comprehensive studies are urgently needed to understand the causes and consequences of intraspecific plant chemodiversity and to elucidate the impacts of such chemodiversity on the interactions of plants with their biotic environment.

Chemodiversity may be enhanced in response to the abiotic and biotic environment, in dependence of phenotypic plasticity (Campbell and Vallano 2018, Gols et al. 2007, Kigathi et al. 2019, Kigathi et al. 2013, Kleine and Müller 2014, Nguyen et al. 2016, van Dam and Baldwin 2001). For example, herbivore or pathogen attack induce local and systemic changes in plant metabolites (Clavijo McCormick et al. 2014, Steppuhn and Baldwin 2007, Travers-Martin and Müller 2007), thereby affecting the chemical profiles of undamaged leaves and even flowers (Bezemer and van Dam 2005, Gonzáles-Megías and Müller 2010, Hoffmeister et al. 2016, Kutyniok and Müller 2012). Differential induction caused by certain organisms can have consequences on interactions of plants with other mutualists or antagonists (Clavijo McCormick et al. 2014, Kumar et al. 2016, Lortzing and Steppuhn 2016, Senft et al. 2017). Surprisingly, many studies on plant-mutualist or plant-antagonist interactions neglect the role of chemical plasticity and more generally the role of intra-individual plant chemodiversity. Moreover, only few researchers take the challenge to study natural variation in perennial non-model plants and particularly in trees.

Most theories on optimal defence production assume that the production of (new) specialised metabolites comes with biochemical costs. These costs are quite difficult to measure directly (Cipollini et al. 2014), though physiological costs for the production of some metabolites have been calculated (Bekaert et al. 2012, Gershenzon 1994). Researchers usually measure fitness proxies, for example, differences in the number of seeds, to estimate direct biochemical costs. Benefits of producing specialised metabolites become evident when plants are in their natural environment, where they interact with a diverse community of herbivores, pathogens and mutualists. The net balance of costs and benefits may determine whether a particular chemical phenotype can survive or even increase in frequency in natural communities. However, the effects of individual specialised metabolites are rarely uniform; a compound deterring many generalist herbivores may be a stimulant for specialists (Calf et al. 2018, Reifenrath and Müller 2008, Schoonhoven et al. 2005). Similarly, floral volatiles attracting pollinators may also make the plant more apparent to herbivores, picking up the same floral scent (Junker and Blüthgen 2010, Unsicker et al. 2009). Consequently, there always will be trade-offs due to contrasting effects of individual metabolites that may simultaneously attract mutualists and antagonists.

Traditionally, the most characteristic taxon-specific specialised metabolites are studied in detail (e.g., specific alkaloids in Solanaceae). More recently, large-scale untargeted metabolic fingerprinting and targeted profiling are used to gain a more complete picture of the overall chemodiversity in plants (Peters et al. 2018). Comparative eco-metabolomics of

different plant species facing an identical challenge (Schweiger et al. 2014b) or plants of one species grown under different conditions (Jansen et al. 2009, Kaling et al. 2015, Schweiger et al. 2014a) combined with multivariate statistical tools (Kuppler et al. 2016, Moritz et al. 2017, Schweiger et al. 2014a) are extremely helpful to gain a better understanding of the specificity of plant chemodiversity and of plant responses towards their environment. Only recently, eco-metabolomics approaches have also been applied to characterise native populations in the field (Clancy et al. 2018, Nagler et al. 2018). Highly sensitive chemical analytical instruments allow us to analyse even small fractions of plant organs such as individual flower parts (Abdalsamee and Müller 2015), phloem sap exudates (Jakobs and Müller 2018, Stolpe et al. 2017a), extrafloral nectar (Lortzing et al. 2016), or trichomes (Schilmiller et al. 2009). Novel strategies for the evaluation and interpretation of metabolomics data are currently developed, for example, using exact mass difference network analyses (MDiN) for the metabolomic description of different genotypes of poplar (Moritz et al. 2017) and chemotypes of *T. vulgare* (Clancy et al. 2018). Such data sets allow disentangling of biochemical mechanisms that modify herbivore interactions (Kaling et al. 2018).

At the molecular level, metabolite diversification can be a result of various mechanisms, including gene duplication and neo-functionalisation, changes in transcriptional regulation or changes in enzymes involved in the biosynthesis of plant metabolites (Moore et al. 2014, Ober 2010). However, the relative contribution of the various mechanisms, including also epigenetic variation (Aller et al. 2018), remains to be elucidated. To explain the large chemodiversity of plants, a few verbal models have been proposed (Firn and Jones 2003, Speed et al. 2012). The current challenge now is to explicitly link the observed chemodiversity with basic evolutionary principles. Thus, mathematical and computational eco-evolutionary models (Wittmann et al. 2013, Wittmann and Fukami 2018) are needed to link genes, enzymes, metabolites and organisms in their ecological environment. Such models will allow us to construct a comprehensive and novel framework for the formation of intraspecific plant chemodiversity and its maintenance over evolutionary time. In addition, we need to define a suitable index similar to the established indices in biodiversity studies that allow us to compare plant chemodiversity within and among species. Given the various expertise needed for these tasks, a thorough understanding of the ecology and evolution of plant chemodiversity can only be achieved through close collaborations of scientists with experimental and theoretical expertise in ecology, chemical ecology, molecular ecology, evolution, (bio)chemistry and statistical and mathematical modelling.

(Joint) preliminary work

Our RU brings together leading experts who have conducted (often jointly) preliminary work in all relevant research fields and are well experienced in their disciplines:

Sybille B. Unsicker (P1) is a chemical ecologist with special interest in the direct and induced defences of woody plant species against their natural enemies such as insect herbivores and pathogens (e.g., Boeckler et al. 2011, Boeckler et al. 2013, Clavijo McCormick et al. 2014, Lämke and Unsicker 2018). Her research combines laboratory

studies in young trees with field studies in mature trees. Methods of classical ecology, analytical chemistry and molecular biology are used to decipher the nature of tree-environment interactions and the underlying mechanisms. Unsicker studied species diversity effects on invertebrates in collaboration with Weisser Unsicker et al. 2006 and plant defences with Schnitzler (Irmisch et al. 2013), Junker (Junker et al. 2011, Junker et al. 2018) and Weisser (Gossner et al. 2014, Kigathi et al. 2019, Kigathi et al. 2013).

Jörg-Peter Schnitzler (P2) is a plant physiologist and biochemist, who is particularly interested in deciphering the metabolic vocabulary of plants and analysing biochemical mechanisms underlying biological functions of plant volatiles (e.g., Kaling et al. 2015, Kaling et al. 2018, Moritz et al. 2017). He combines molecular biological (transcriptomics), biochemical (metabolomics) and ecophysiological (gas exchange, spectral sensors) techniques to analyse the impact of abiotic and biotic factors on plant fitness, with a focus on volatile compounds and stress-induced metabolites. Schnitzler uses new tools such as mass difference enrichment analysis that incorporate unidentified metabolites in chemotypic description and applied this technique with Weisser (Clancy et al. 2016, Clancy et al. 2018). He also worked with Unsicker on plant defences (see above).

Anke Steppuhn (P3) is a molecular ecologist, who investigates plant defence strategies against herbivore feeding and egg deposition. She aims to understand the strategies of plants used to fend off herbivores, costs and benefits involved in defence under different environmental conditions and how plants perceive herbivore attack (e.g., Lortzing and Steppuhn 2016, Lortzing et al. 2017, Steppuhn et al. 2008). Her research focuses on solanaceous plants and involves bioassays, ecological field experiments, chemical analyses of plant metabolites (targeted and untargeted) and molecular approaches, including transcriptomics, sequencing and gene silencing. Steppuhn and van Dam have been working closely together on *S. dulcamara* Lortzing et al. 2016, Nguyen et al. 2018, Nguyen et al. 2016).

Nicole M. van Dam (P4) is a molecular ecologist, studying the chemical and molecular mechanisms underlying interactions between plants and their environment, especially herbivores and higher trophic levels. She has a long-standing expertise in the metabolite analysis of above- and belowground herbivore-induced defence responses (e.g., Bezemer and van Dam 2005, Calf et al. 2018, van Dam et al. 2010, van Dam 2009). For the analyses of plant samples, she uses a combination of molecular (transcriptomics) and ecometabolomic approaches (e.g., Peters et al. 2018, together with Müller), combined with manipulative experiments to assess the ecological relevance. Van Dam and Steppuhn have been working closely together on *S. dulcamara* (see above) and van Dam contributed to the meta-study by Junker (Junker et al. 2018).

Caroline Müller (P5) is a chemical ecologist, who investigates the role of natural products involved in communication between organisms. She is particularly interested in the inter- and intraspecific variation in plant responses to environmental challenges, in tissue-specific analysis of metabolites with special focus on phloem sap and in the role of factors modulating plant-antagonist interactions (e.g., Jakobs et al. 2019, Schweiger et al. 2014a, Schweiger et al. 2014b, Schweiger et al. 2014c, Tewes et al. 2018). Müller combines

chemical-analytical tools with bioassays and behavioural studies to elucidate the ecological and evolutionary basis of species interactions. She collaborates with Eilers and Weisser in studying the role of chemodiversity in *T. vulgare*-insect interactions and explored the use and challenges of eco-metabolomics with van Dam (Peters et al. 2018).

Elisabeth J. Eilers (P6) is an ecologist, who investigates chemo-ecological aspects of mutualistic and antagonistic plant-insect interactions (e.g., Kallenbach et al. 2014, Henselek et al. 2018, Eilers et al. 2015). She therefore acquired skills in designing and executing field and laboratory experiments, chemical analysis (particularly plant volatiles above- and belowground), neuro-ethological methods (including bioassays), statistical analysis of complex data as well as plant and insect rearing methods. Eilers is particularly interested in the role of chemodiversity in economically and ecologically relevant plants that can explain plant invasiveness and species interactions. Eilers and Müller have already collected seeds of *T. vulgare* in the field, and Eilers is currently chemotyping the offspring plants to be used for the projects of the RU.

Wolfgang W. Weisser (P7) is an insect ecologist, who is interested in biodiversity and in multitrophic interactions at the population, community and ecosystem level. He studies interactions between plants, herbivorous insects and their natural enemies as well as mutualists, and investigates the involvement of plant volatiles in these interactions, using *T. vulgare* as model (Clancy et al. 2016, Clancy et al. 2018). Weisser has worked extensively on the role of plant diversity for plant-arthropod interactions. In the framework of this RU, he is especially interested in the role of plant chemodiversity for structuring arthropod communities. Plant material and expertise on *T. vulgare* is exchanged with Müller and Eilers. Weisser and Unsicker have close collaborations on various projects (see above).

Andrea Bräutigam (P8) is a computational biologist with special interest in network analysis and modelling. Her research focusses on elucidating the molecular composition of complex traits and their evolution (e.g., Bräutigam et al. 2014, Bräutigam et al. 2011a, Bräutigam et al. 2011b, Ramírez-González et al. 2018). Bräutigam combines bioinformatic and biostatistic methods with biochemical and physiological measurements. Within the RU, she is particularly interested in the effects of selection on the molecular level that explain the role of chemodiversity in plant-herbivore interactions. Bräutigam received terpenoid data on chemotyped plants and their offspring and chemotyped plant material from Müller and Eilers for RNA-seq analysis to develop the biochemical function of terpene synthases in *T. vulgare*.

Meike J. Wittmann (P9) is a theoretical biologist broadly interested in theoretical ecology and population genetics, and in particular in the interplay between ecological and evolutionary processes (e.g., Wittmann and Fukami 2018, Wittmann et al. 2013, Wittmann et al. 2017). Recently, MJW has focused on building and analysing eco-evolutionary equation-based models and simulations for the maintenance of species diversity and genetic diversity, for example, by spatial and temporal variation in selection pressures. She is eager to apply this experience to the new challenge of modelling plant chemodiversity. To make sure that the empirical results and theoretical models within the RU can be

fruitfully connected, Wittmann already discussed in detail the design of the common garden and cafeteria-style experiments planned in the RU, in particular with Müller and van Dam.

Robert R. Junker (P10) is an evolutionary ecologist, who focuses on plant-animal and plant-bacteria interactions. He investigates how volatile compounds affect the diversity and interaction patterns of insects and bacteria and how these organisms alter the phenotype of plant species (e.g., Junker and Blüthgen 2010, Hoffmeister et al. 2016, Kuppler et al. 2016). His expertise in performing field and lab experiments is complemented by pronounced skills in the development and application of statistical approaches. Recently, Junker proposed statistical analyses of the diversity of chemical phenotypes (scent bouquets) that consider the biosynthesis of the involved compounds. Junker collaborated with Unsicker and van Dam for a meta-analysis on the structure of chemical phenotypes (Junker et al. 2018).

Project-related publications

Principle investigators highlighted

Bräutigam A , Mullick T, Schliesky S, Weber APM (2011) Critical assessment of assembly strategies for non-model species mRNA-Seq data and application of next-generation sequencing to the comparison of C3 and C4 species. J Exp Bot 62: 3093-3102

Calf OW, Huber H, Peters JL, Weinhold A, **van Dam NM** (2018) Glycoalkaloid composition explains variation in slug resistance in *Solanum dulcamara*. Oecologia 187: 495-506.

Clancy MV, Zytynska SE, Moritz F, Witting M, Schmitt-Kopplin P, **Weisser WW**, **Schnitzler JP** (2018) Metabotype variation in a field population of tansy plants influences aphid host selection. Plant Cell Environ 41: 2791-2805

Clavijo McCormick A, Irmisch S, Reinecke A, Boeckler GA, Veit D, Reichelt M, Hansson BS, Gershenzon J, Köllner TG, **Unsicker SB** (2014) Herbivore-induced volatile emission in black poplar: regulation and role in attracting herbivore enemies. Plant Cell Environ 37: 1909-1923

Eilers EJ , Pauls G, Rillig MC, Hansson BS, Hilker M, Reinecke A (2015) Novel set-up for low disturbance sampling of volatile and non-volatile compounds from plant roots. J Chem Ecol 41: 253-266

Geuss D, Stelzer S, Lortzing T, **Steppuhn A** (2017) An ovicidal response of *Solanum dulcamara* to insect oviposition. Plant Cell Environ 40: 2663-2677

Jakobs R, Schweiger R, **Müller C** (2019) Aphid infestation leads to plant part-specific changes in phloem sap chemistry, which may indicate niche construction. New Phytol 221: 503-514

Junker RR, Kuppler J, Amo L, Blande J, Borges R, **van Dam N**, ..., **Unsicker SB**, et al. (2018) Covariation and phenotypic integration in chemical communication displays: biosynthetic constraints and eco-evolutionary implications. New Phytol 220: 739-749

Weisser WW , Roscher C, Meyer ST, Ebeling A, Luo GJ, Allan E, et al. (2017) Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions. Basic Appl Ecol 23: 1-73

Wittmann MJ, Fukami T (2018) Eco-evolutionary buffering: rapid evolution facilitates regional species coexistence despite local priority effects. Am Nat 191: E171-E184

Objectives, concept and approach

Common objectives of the RU

In the envisaged RU, we will investigate the ecology and evolution of intraspecific, including intra-individual, chemodiversity in three focal plant species. Combining field and laboratory studies with untargeted metabolomics and targeted metabolite profiling as well as genetic tools, statistical data mining and modelling, we want to elucidate the **ecology and evolution of intraspecific chemodiversity of plants.**

Our **central questions** are:

1. How does plant chemodiversity vary across levels, i.e. within individuals, among individuals within populations, and among populations?
2. What are the ecological consequences of intraspecific plant chemodiversity?
3. How is plant chemodiversity genetically determined and maintained?

By addressing these questions, we aim to provide a scientific basis for understanding the role of intraspecific plant chemodiversity in natural plant communities, which may have important implications for pest control in crops, ecosystem functioning and nature restoration. We will take a combined experimental and theoretical approach, whereby ecological field and laboratory studies will be complemented with chemical and genetic analyses as well as mathematical modelling (Fig. 1).

Within the first funding period, we aim to:

- develop a conceptual framework to describe chemodiversity, adopting and comparing common biodiversity measures for application to plant chemodiversity
- characterise the constitutive and induced plant chemodiversity within individuals, within populations and among populations with spatio-temporal resolution
- assess the effects of plant chemodiversity on herbivores with different feeding mode as well as pollinators and the contribution of phenotypic plasticity therein
- describe potential trade-offs between costs and benefits of plant chemodiversity on a community level

- identify and characterise genes coding for biosynthetic pathways relevant for plant-insect interactions and link chemodiversity to genetic features
- test whether patterns of chemodiversity in the three species support or contradict different hypotheses that aim to explain chemodiversity

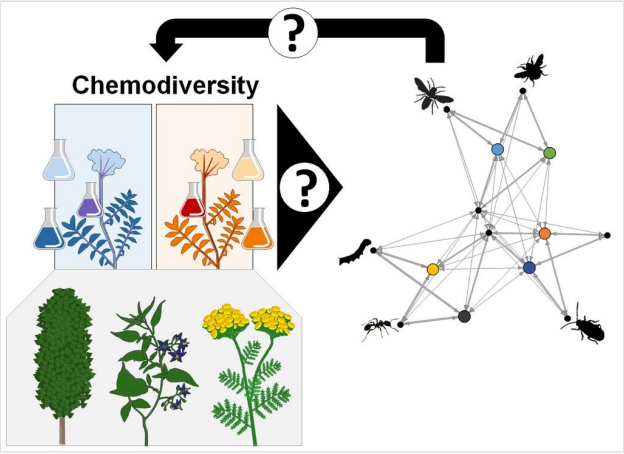

Figure 1. doi

Conceptual framework of the proposed RU on the ecology and evolution of intraspecific plant chemodiversity. We will study chemical variation in different plant parts (flowers, nectar, pollen, leaves, phloem sap; roots will be included in a potential second funding period), among plant individuals within populations and among populations (*left*) as well as consequences on the plant-associated community (*right*) over space and time. The projects will focus on the tree *Populus nigra* and the herbs *Solanum dulcamara* and *Tanacetum vulgare* (lower panel, from left to right).

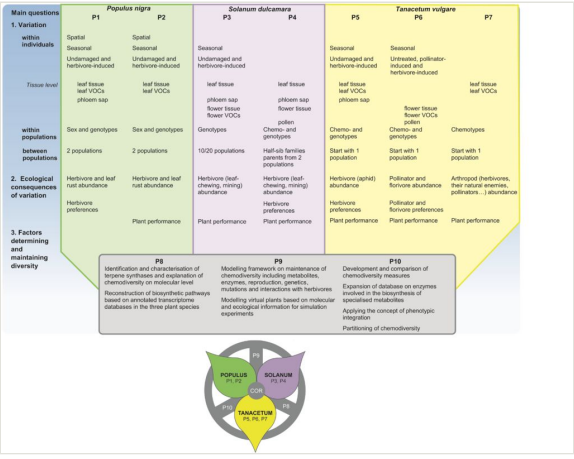

Figure 2. doi

Parallel research approaches in individual project parts and synergies of this RU.

- develop a new eco-evolutionary modelling framework for intraspecific plant chemodiversity
- generate specific data to parametrise the theoretical models

The overall aim of an envisaged second funding period is to implement the insights gained in the first phase into a broader context by:

- extending the geographic scale of study populations in all three species to comparatively asses the role of the breadth in environmental conditions accompanying their wide distribution
- including belowground intraspecific plant chemodiversity and effects on root herbivores
- investigating the causes and consequences of plant chemodiversity in more complex (natural) settings including microorganisms and higher trophic levels
- exploring the role of plant chemodiversity from the herbivore and pollinator perspective in more detail (e.g., in terms of counter-adaptations)
- developing genomic resources for the three study species such that chemodiversity can be linked to specific gene (and potentially epigenetic) variation and positioning in the genome
- incorporating more genetic realism into mathematical models for plant chemodiversity

Expected benefits of collaboration within the RU

This RU brings together a highly motivated, well-integrated and diverse group of scientists. We share a common vision on the ecology and evolution of plant chemodiversity. As a group, we can test hypotheses suggested by the currently available models explaining chemodiversity in parallel at multiple levels of complexity and synthesise the data to form well-supported conclusions. For example, the RU as a whole will be able to resolve if one of the key predictions from the screening hypothesis, *i.e.* that “plants maximise chemodiversity”, applies in the three study species. The combined expertise in this RU brings us in the unique position to thoroughly test this hypothesis in three distinct natural plant systems.

At the same time, each member has a unique area of expertise to generate the necessary synergies for a successful implementation of this joint project. Together, the competence of the applicants covers all methods necessary to address the questions and hypotheses of the individual projects as well as the data synthesis. Integrating field studies and classical ecological experiments with –omics techniques, bioinformatics and theoretical modelling will foster the exchange of knowledge and skills among RU members. Effective knowledge exchange will be ensured by regular meetings of all RU members and by lab visits of the young investigators (PhD students, postdoc). By these means, the young investigators will have access to a multifaceted method toolbox and expertise, which individual supervisors cannot offer.

For example, bioinformatic analyses of molecular data will be particularly supported by Bräutigam, who is an expert in transcriptome analyses and supervised machine learning, to study complex traits along evolutionary trajectories and to characterise traits at the molecular level (Bräutigam et al. 2014, Bräutigam et al. 2011a, Bräutigam et al. 2011b, Ramírez-González et al. 2018). Furthermore, the RU will highly profit from the mathematical modelling expertise of Wittmann (Wittmann et al. 2017, Wittmann and Fukami 2018), who will provide a theoretical framework for the evolution and maintenance of plant chemodiversity. Bräutigam and Wittmann were successfully recruited in 2017 for a W2 professorship and a Junior-professorship with tenure option, respectively, at Bielefeld University. Their expertise complements interests by present researchers at Bielefeld University (Müller, Eilers), which will facilitate easy collaboration particularly among these groups.

Various metabolomics approaches have been developed in several labs involved in the RU. For instance, the collection of volatiles using push pull systems or passive trapping by polydimethylsiloxane (PDMS) tubes is well established by various PIs (Clavijo McCormick et al. 2014, Hoffmeister et al. 2016, Jakobs and Müller 2019, Kallenbach et al. 2014, van Dam et al. 2010). Large-scale untargeted metabolomics using liquid and gas chromatography (LC and GC) coupled with mass spectrometry (MS) combined with multivariate statistics are routinely applied in various labs of the RU [Müller (Schrieber et al. 2019, Schweiger et al. 2014b), Schnitzler (Kaling et al. 2015, Moritz et al. 2017), van Dam (Calf et al. 2018)]. By exchanging experience on advantages and disadvantages of individual methods and by intensive knowledge transfer, we will establish and optimise methods that will be commonly used in all respective groups (e.g., ring trial, Fig. 3). This will allow for a better comparison of the individual data (e.g., for the synthesis) as well as benefit the entire chemical ecology research community.

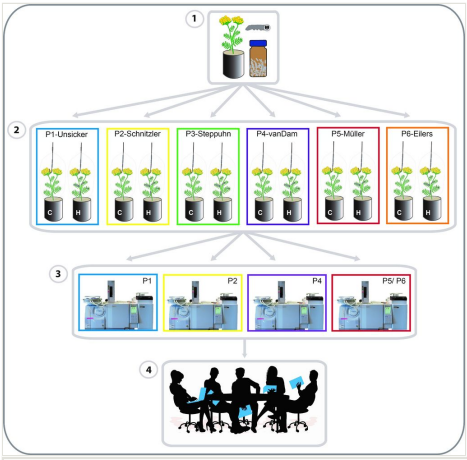

Figure 3. doi

Scheme of the collaborative ring trial within the RU. For details see text. C – control; H – herbivore-treated.

A challenge in the analysis of plant chemodiversity is the huge number of not yet chemically characterised metabolites. Given that concurrent identification and quantification of entire plant metabolomes is not possible to date, analytical tools such as mass difference networks (MDiN) and mass difference enrichment analysis (MDEA) can expand our capacity to analyse mass spectrometry data. These tools are established by Schnitzler (Kaling et al. 2018, Moritz et al. 2017) and will be made available for the RU.

Phloem sap collection by laser stylectomy or using ethylenediaminetetraacetate and analysis of the exudates is well established in the Müller group (Kuhlmann and Müller 2010, Schweiger et al. 2014c, Stolpe et al. 2017a). Students of the groups performing phloem sap collection and analysis (Müller, Steppuhn, Unsicker, van Dam) will be trained in the Müller lab and apply the methods to their research questions. A comparison between nectar and phloem sap chemistry will be of particular interest (as in Lortzing et al. 2016). For analysing structural sugar isomers in phloem exudates and nectar, a Trapped Ion Mobility LC-MS-MS (timsTOF^{TM}, Bruker) is available in the Unsicker group.

Investigating the role of intraspecific plant chemodiversity for herbivore resistance and its importance in shaping trade-offs among metabolites in affecting antagonists and mutualists is a common aim in this RU. Investigating this topic comprehensively will be enabled by the complementary expertise of different PIs on plant resistance mechanisms (Calf et al. 2018, Kigathi et al. 2013, Müller 2008, Steppuhn and Baldwin 2007, Steppuhn et al. 2008). Similar set-ups for common garden experiments with different levels of intraspecific chemodiversity will be used in all empirical projects to disentangle genotype-phenotype relationships and effects of plant chemodiversity on insect communities. In parallel, the molecular basis of terpene diversity will be evaluated by Bräutigam. For example, a set of candidate genes for single nucleotide polymorphism (SNP) analysis of a *T. vulgare* field population is currently developed. These data can be used for population genetic analyses of other *T. vulgare* individuals.

The ecology and evolution of intraspecific plant chemodiversity can only be understood by studying the consequences of this diversity in the field and in ecologically relevant experiments. Thus, a very important feature of the RU is to study the effects of chemodiversity in the field. Most PIs have extensive experience in studying interactions between plants and other organisms in common gardens and the field.

Finally, **the integration of data (synthesis)** is one of the key goals of the current RU. Combining the metabolic data from the different plant species and tissue types, we aim to identify patterns of co-variation in the composition of metabolites that can be aggregated in **phenotypic integration** values across an organisational hierarchy. Junker has been very successful in bringing together data from various researchers to study biosynthetic constraints and eco-evolutionary implications of the co-variation and phenotypic integration in chemical communication displays (Junker et al. 2018) and will lead this part of the RU.

Expected key results in the short- and medium-term

We focus on three plant species differing in life-history traits to address the common key questions of this RU on the ecology and evolution intraspecific plant chemodiversity. The common approach used in this RU will strongly contribute to our understanding of how plant chemodiversity varies across different levels within selected plant species and how such chemodiversity affects interactions with other antagonistic and mutualistic organisms. We will determine how biotic factors influence the biosynthesis of specialised metabolites and reveal patterns of co-variation. In conclusion, **we will gain novel insights on several levels of intraspecific plant chemodiversity and its functionality in interactions with other organisms.**

Long-term goals

In the long-term, we aim to study additional layers of intra-individual differences. This will be achieved by including research on the chemodiversity of roots and interactions with belowground herbivores as well as with symbiotic or pathogenic microorganisms. Moreover, populations from a broader geographic range differing in selection histories will be included. We also intend to investigate the role of epigenetics in chemodiversity as a possibly important mechanism for the adaptation of perennial plants to varying herbivore pressures. Studying three plant species with different life-history traits in detail will allow us **to refine models explaining evolutionary origins of chemodiversity**.

Work programme including proposed research methods

For this RU on chemodiversity we propose to combine a variety of different experimental approaches with state-of-the-art analytical techniques and mathematical modelling approaches to characterise metabolic phenotypes with high resolution mass spectrometry. At the same time, we aim to develop a clearer definition of the term chemodiversity, whereby chemodiversity can be found at different hierarchical levels. This is needed to explicitly link metabolic patterns to community dynamics and biodiversity.

Research framework

Study systems: We focus our research on three study species that are ideal to address our research questions, *P. nigra*, *S. dulcamara* and *T. vulgare*. All three species exhibit a high degree of chemodiversity and naturally occur in Northern and Central European ecosystems including Germany. Although none of these species are classical model plants in molecular ecology, they fulfil essential characteristics for studying the eco-evolutionary relevance of chemodiversity also under natural selective regimes.

The three species selected for our projects have several features in common, which will allow us to transfer concepts and to determine more general principles. They are perennial and can be cloned easily, which is important for obtaining sufficient replicates and testing phenotypic plasticity while controlling for genetic background. Furthermore, all three species have been introduced to other continents. This widespread distribution will allow us

in the long-term to compare populations with different selection histories on a large geographic scale. Most importantly, the three species have received attention based on their interesting metabolites, e.g. in the field of pharmaceutics (Hage and Morlock 2017, Kumar et al. 2009, Mitich 1992). Regarding their volatile bouquets, they share some metabolite classes such as terpenoids. With respect to their species-specific metabolites, *P. nigra* is mainly characterised by phenolics (Boeckler et al. 2011), *S. dulcamara* by glycoalkaloids (Eich 2008) and *T. vulgare* by mono- and sesquiterpenoids (Keskitalo et al. 2001). Based on the literature and our own experience, we expect an increasing level of diversity in common classes of metabolites such as terpenoids from *P. nigra* up to *T. vulgare.* A gradient in dependence on animal pollination is also found across the three species, with *P. nigra* being wind-pollinated (Düll and Kutzelnigg 2016), *S. dulcamara* mainly pollinated by bumblebees (De Luca and Vallejo-Marin 2013), and *T. vulgare* being visited by various pollinator species (Düll and Kutzelnigg 2016). This gradient in pollination systems opens up the interesting opportunity to study the variation and extent of chemodiversity of flower volatiles. In parallel, pollen defence chemodiversity will be investigated, because flower rewards also need some protection against robbery. Another important point for the molecular exploration is the fact that the three species are diploid. First molecular data and resources on all three species are available or currently generated within our RU (transcriptomes of all three species).

Using a common experimental design, leaf volatiles will be measured in the three study species applying the same methodology [PDMS tubes (Kallenbach et al. 2014)]. Measurements will be taken at three representative time points over the year, which may differ according to the species' biology (**P1–P7**). This approach will enable us to test how levels of chemodiversity depend on the life-history and growth form of the species (**P10**). Moreover, untargeted finger-printing and targeted analyses of characteristic defence metabolites will be performed using high performance LC quadrupol time of flight MS (LC-QTOF-MS) and GC-MS. MDEA will be used for in depth data evaluation (supported by **P2**). Furthermore, the associated herbivore and pollinator community as well as damage patterns will be recorded in parallel common garden experiments with plots of single and mixed chemotypes, allowing us to detect correlations between plant chemodiversity and herbivores at both spatially and temporally relevant scales. Metabolic data will be complemented with transcriptomics data in all species (**P1–P2**, **P4**, **P8**). We will also generate data to identify properties of biosynthetic pathways (**P8–P10**) and develop mathematical models as well as 'virtual plants', which can then be subjected to simulation experiments and comparative analyses (**P9**). The individual project parts and synergies are depicted in Fig. 2.

Milestones and synergies

The expected benefits of collaboration (Expected benefits of collaboration within the RU), expected key results in the short- and medium-term (Expected key results in the short- and medium-term), and the long-term goals (Long-term goals), are outlined above and summarise our milestones and synergies. As an additional overarching milestone we need to reach a proper definition of 'chemotype' and 'chemodiveristy'. As a working definition, we

define a chemotype as a group of plants within a species, which can be distinguished according to the distinct and mostly heritable relative composition of compounds belonging to one prominent class of specialised metabolites, e.g., glycoalkaloids or terpenoids (Linhart et al. 2005, van Leur et al. 2006). Chemodiversity may be calculated by applying various indices derived from biodiversity research (Morris et al. 2014). Often, the Shannon index is used, which considers the number of occurring metabolites and their abundance (Wolf et al. 2012, Tewes et al. 2018). Moreover, as in biodiversity, chemodiversity can be measured at different levels, with α-diversity considering the number and abundance of chemical metabolites produced by each individual, β-diversity measuring the difference between two profiles of specialised metabolites and γ-diversity considering the overall chemodiversity in a population or region. Within the first year of the RU, we will utilise the various data sets produced in our working groups to evaluate the suitability of different measurements for chemodiversity (**P1–P10**). A workshop on this topic will be held in the first year. The goals will be to develop the most comprehensive conceptual definition of 'chemodiversity' and to apply the most meaningful measure of chemodiversity to all data sets. Developing a unifying concept of chemodiversity will be of importance for the entire chemical ecology community.

Moreover, for a synthesis of the various data from the individual projects, we aim to calculate the phenotypic integration of plant metabolites across an organisational hierarchy (mostly second year). The empirical data sets produced by the members of the RU in their individual projects (**P1–P8**) will provide the scientific basis to investigate the biosynthetic and genetic organisation of chemical trait co-variation and to evaluate the functional importance of phenotypic integration in plant metabolites (**P10**). In order to discriminate among functional, genetic, developmental and environmental causes of co-variation, phenotypic integration and modularity (Klingenberg 2008), we will analyse patterns of variation and co-variation at the level of an individual, at the population and at the species level. In the modelling project (**P9**), we will obtain a conceptual integration of genetic architecture of biosynthetic pathways with ecological interactions between plants and their herbivores and pollinators. Drawing on expertise and data from the empirical projects, the models will be parameterised for each of the three study systems. This parameterisation allows us to make qualitative, and in some cases also quantitative, predictions for chemodiversity patterns (i.e., patterns of allele frequencies and genotype-phenotype maps) to be expected in the three study species (third year). These models will provide insight into potential evolutionary explanations for plant chemodiversity and will allow generating new hypotheses to be experimentally tested in a potential second funding period.

In conclusion, our combined approach will substantially contribute to our understanding of how plant chemodiversity varies across different levels, how such chemodiversity affects interactions with other antagonistic and mutualistic organisms and how it is maintained.

Research data and knowledge management

In addition to routine communication (by e-mail, phone and Skype), we will use the following means to ensure an efficient and stimulating exchange of knowledge:

Internal data sharing platform and data management: We have established a project share box in Sciebo (cloud storage platform of research institutions in North Rhine-Westfalia, guest status for members of the RU *via* Bielefeld University). This share box serves as the platform for data integration and management and for the exchange of method protocols within our group.

Public data repositories: We will create a detailed data management plan that will address all questions regarding data capture, storage, back-up, documentation, publication and long-term archiving of data collected at the beginning of this project. The Research Data Management Competence Center of Bielefeld University will assist the RU in developing procedures for Findable, Accessible, Interoperable, and Re-usable (FAIR) data during the project. Raw data of ecological and environmental parameters will be made available by publication with digital object identifiers (DOI). Modelling and simulation code will be placed under an open source licence. For both data and code, we will use repositories such as dryad or figshare, institutional repositories, or upload the material directly as supplementary information. Metabolomics and RNA-seq data will be uploaded to the corresponding repositories. MetaboLights is an open-access database for metabolomics experiments assigning studies a unique accession number as publication reference. Adhering to best practices in metabolomics data sharing will be done in collaboration with the de.NBI MASH project (Steffen Neumann, IPB Halle). Steffen Neumann offered his support to the RU. Prior to the upload of RNA-seq data to the European Nucleotide Archive (ENA) or gene expression omnibus (GEO), data will be stored in a network file system with automated back-ups (Bräutigam). Thus, all data and code connected to publications will be shared according to FAIR principles, available long-term and be quotable by DOIs.

Potential impact on the research area and local environment

All PIs involved in the RU are leading experts in their field and their home institutes have a high scientific reputation. Several researchers within the envisaged RU collaborate already successfully as evidenced by joint publications (see (Joint) preliminary work). Between and among researchers, the discussions leading to the RU have spawned collaborative efforts, which will strengthen over time, bringing the researchers and their institutions closer together and leading to fruitful collaboration. With this RU, we expect to become nationally and internationally visible as a scientifically strong consortium working on intraspecific plant chemodiversity. At the same time, we expect that our consortium will have a significant impact on the international community of chemical ecologists by building bridges among research disciplines, study organisms and study levels to achieve common overarching goals. This will happen when RU members present their work at international conferences, *via* publications in international journals including special issues on chemical diversity edited by the applicants, and by means of our workshops with national and international guests.

Measures to advance research careers

Early career researchers

The proposed RU includes several young scientists as PIs (Eilers, Steppuhn, Unsicker, Wittmann), who made already major contributions in their fields. Eilers joined the group of Müller in 2016 and is building up her independent research while pursuing a habilitation. Steppuhn was promoted to a W2 position (non-permanent) at Free University of Berlin in 2017. Unsicker is currently working on her habilitation. Wittmann started a position as Jun. Prof. (tenure-track) in 2017 at Bielefeld University. A successful participation in an internationally respected RU will increase the visibility of these young researchers, advance their careers and increase their chances to be tenured. Wittmann will be the Deputy Speaker of the RU, offering her the opportunity to gain experience in leading a large collaborative DFG-funded project.

Doctoral programmes

The young investigators employed in this RU will profit from structured doctoral programmes established at all involved universities, iDiv, Helmholtz and MPI-CE. Furthermore, they will choose a second mentor who is a PI in the RU but not their direct supervisor. The mentee will meet with her/his second mentor at least twice a year and will discuss the ongoing work, planning of experiments and manuscripts as well as career prospects. Financed by the travel budgets of the individual projects (**P1–P10**), the young investigators will be enabled to present their research to the scientific community and to network at national and international conferences.

Gender equality, career and family

Our PIs can serve as role models in various ways. Seven out of 10 (70 %) of our PIs are female and can serve as role models of successful female scientists. Several of our PIs (see CVs) can demonstrate to young scientists that it is possible to raise children and be successful in academia. When hiring young investigators for the projects, a balanced gender ratio will be strived for. Female junior scientists will be encouraged to participate in mentoring programmes and soft skill workshops adapted to the particular challenges of women in science such as *movement* (Bielefeld University) or *Minerva-FemmeNet* (MPG). All involved institutions have policies in place to ensure gender equality and family-friendly work conditions. Additionally, funds are requested for flexible childcare and home office supplies for all scientists with care tasks (for details see Project-specific Workshop Module). Furthermore, PIs will raise their awareness about the diversity of students (different genders, cultural backgrounds, etc). They will promote equality in chances and work against discrimination and under-representation by taking part in trainings offered by their institutions and staying informed *via*, e.g., https://www.working-between-cultures.com.

National and international cooperation and networking

The networking mechanisms described above (Expected benefits of collaboration within the RU) and the planned retreats and workshops (see below) will establish our RU as a national research consortium leading this field. National and international scientists will be invited to our workshops to stimulate discussions about chemodiversity in our scientific community and to encourage our young investigators to start building scientific networks. Furthermore, participation in international conferences such as the Gordon Research Conference on Plant-Herbivore Interaction as well as meetings with a focus on plant ecology and evolution will stimulate international cooperation and networking.

Coordination

Description of how joint objectives and the joint work programme will be implemented in the coordination project

The coordination project and thus the speaker of the RU will facilitate the communication (including data management) and cooperation among the geographically dispersed groups and their research teams. The communication within the RU is based on regular meetings of all members but also on exchange between individual groups. For exchange between all groups and the scientific community, retreats and workshops will be coordinated as listed below. Exchange among individual groups will be mostly realised by mail, phone and skype or potentially video conferences. Webinars may enable virtual participation in seminars of general interest. For method standardisation, a ring trial for volatile collection is planned (see Coordination Module, Fig. 3), which will be coordinated in the central project. Similar ring trials or method adjustment will also be implemented for other techniques, e.g., metabolomics.

Anticipated total duration of the project

Three years.

Requested modules

Coordination Module

For administrative assistance as part of coordination, we apply for one service assistant (TVL E8, 50 %) for the speaker of the RU, Müller. The service assistant will provide the services for the administrative management of the RU, manage the central budget, administer the common website and shared data platform (Research data and knowledge management) as well as help in scheduling, organising and implementing the retreats and workshops.

Furthermore, a student assistant (10 h/week for 36 months) is requested who will be involved in the following three tasks:

- Structuring of data and metadata format for data to be collected by all groups and stored centrally; support in data upload and management (student with bioinformatics background)
- Support in preparation and cultivation of *T. vulgare* clones used in the projects **P5–P8**
- Coordination of ring trial (preparation of material, comparative data analysis):

At the beginning of the RU, we plan a ring trial of volatile collection by PDMS and analysis by thermodesorption (TD)-GC-MS. Clones of *T. vulgare* will be distributed by **P5** and **P6** to different groups (Fig. 3). Each of these groups will receive a set of PDMS tubes and a set-up for volatile collection. Volatiles will be collected in technical replicates from the plants on PDMS tubes (control plants and plants infested with 3rd instar larvae of the generalist *Spodoptera littoralis*, provided by **P1**). These tubes will be sent by each group to the groups with a TD-GC-MS. The volatile data will then be compared. This work will help us to clarify and validate, at the beginning of the project, how comparable analyses among our labs are. It will also train the PhD students involved in the projects in volatile collection and methodological challenges. We expect to jointly publish the data of this ring trial as a short communication in an open access journal.

Metabolomics platforms exist in several groups. The PIs of the individual projects have made individual agreements where they will run their analyses for an efficient division of resources and instrument time over the various labs. The PIs leading the platforms will be in regular exchange of expertise, methods and protocols. For ready comparison of metabolome data gathered by the different groups we will establish common sample preparation and measurement methods, as far as possible. Moreover, Schnitzler in collaboration with Philippe Schmitt-Kopplin (Helmholtz Zentrum München) established novel analytical methods such as mass difference networks, which can be applied on metabolomics and transcriptomics data sets. Schmitt-Kopplin will offer his expertise for data analysis in various projects.

RNA-seq analysis: An RNA-seq platform followed by single nucleotide variation (SNP)-based population analyses will be offered by Bräutigam. The platform includes the analytical set-up for quantitative transcriptome analyses of non-model species and the analytical set-up for transcriptome assembly, SNP discovery and quantification. We will offer a three-day RNAseq workshop ("*Hackathon*") to effectively transfer knowledge within the RU.

Advisory Board: We will set up an Advisory Board of three experts in the field with international reputation. These Board members will be invited to the workshops and may potentially host a young investigator for a lab visit and training in their lab.

Lab exchange of young investigators: The young investigators will be encouraged to visit other labs of PIs of the RU and beyond (e.g. potentially members of the advisory

board) to be trained in methods that are important for their projects. For these visits, we apply for a lump sum of 15,000 €.

Network Funds Module

We aim to share metabolite standards and buy new standards, which will be distributed to all analytically working groups, to expand our databases needed for metabolite identification. To realise this goal we apply for a 10,000 € lump sum.

Gender Equality Measures in Research Networks Module

All participating institutions have explicit policies in place to ensure gender equality and family-friendly work conditions. These existing support structures and services will be strengthened by the following provisions:

- Flexible childcare and home office supplies for all scientists with care tasks
- Childcare during conferences or other events organised by the RU
- Flexible funds for, e.g., compensation for the absence of a RU member during pregnancy, care tasks for sick family members or parental leave
- Workshop on how to train a wide diversity of students and promote equal opportunities at all levels
- Specific communication trainings empowering young scientists to deal with implicit bias and micro-aggression, e.g. Active Bystander training by Scott Solder (http://www.scottsolder.com/)

For these measures, we apply for a lump sum of 15.000 € p.a.

Project-specific Workshop Module

Yearly retreats: All PIs and the young investigators involved in the projects will meet at least once per year for a 1-2 day retreat, which will be combined with a workshop. These retreats will be used to align the projects and to synthesise the most important findings and achievements of the RU. The young investigators will present their data to allow for feedback of other members of the RU and invited guest speakers. Guest speakers will give valuable input to the RU by reporting on adjacent topics. Furthermore, we will organise a “science speed dating” event where each young investigator talks for 5 min to each guest speaker (of course in the hope that longer conversations will follow) to improve networking skills and to get advice on academic careers. The young investigators will be encouraged to organise an own programme block directly before or after the retreat and workshop, in which they may exchange without their PIs and organise personal or scientific development courses depending on how they choose to allocate their time and financial support. For these young investigator modules, we apply for a lump sum of 5,000 € p.a. Having responsibility for the budget and the content will allow the young investigators to develop responsibility and deepen their scientific interactions while at the same time the self-developed programmes will address their actual needs.

Workshop 1: In the first year, we plan to organise a workshop on the topic “Plant Chemodiversity” to discuss the most useful chemodiversity measurements and indices developed by project **P10** based on data provided by **P1–P7**. This workshop will be hosted by Junker in Marburg and international experts in biodiversity, chemical ecology and handling of complex metabolomic datasets will be invited.

Workshop 2: In the second year, the workshop will focus on “Evolution of Chemodiversity” and will be chaired by Wittmann. The workshop will take place in Leipzig at the German Centre for Integrative Biodiversity Research (iDiv) and co-organised by van Dam and members of her group. Guest speakers will be invited to discuss with us causes and consequences of chemodiversity.

International Workshop 3: A third workshop aims to discuss the findings of the RU in an international context. The “Synthesis” of our findings will be presented and discussed with international experts and we aim to write a joint paper on “The ecology and evolution of plant chemodiversity”. The workshop will be held at the Centre for Interdisciplinary Research (ZIF) of Bielefeld University and will be co-hosted by Eilers and Müller. At least 15 national and international experts in this field will be invited. To co-fund this workshop, we will also apply for funding at the ZIF.

Hackathon: In addition to the workshops, a “Hackathon” will be offered in the second year, which will be organised by Bräutigam, with input from Schnitzler and van Dam. All members of the RU will gather insight in transcriptome assembly, quantification and analyses for application in ecological and evolutionary concepts. The participants will meet in a conference room equipped with computers directly linked to the compute cluster at CeBiTec, Bielefeld University, and direct data access. Jointly, we will handle our data, share methods, scripts, programmes and ideas, pursue wild ideas and push our projects forward. This hackathon will foster interactions among the researchers which will strengthen the collaborations and give us room to try new and unusual approaches and share and develop new ideas.

Public Relations Module

A website will be established to advertise the general aims of our planned RU and to briefly introduce the aims of the individual projects potentially interesting to the scientific community as well as the public. The content will be regularly updated with current information, results, ideas and data from our projects. The estimated costs for this website will be around 3,000 €, to engage a professional to ensure a functional and attractive website that increases visibility. Dissemination of information to the group and the public will also make use of Twitter. Press releases will be issued on fundamental results *via* the press offices of the institutions.

In addition, we will train the PIs and especially the young investigators in science communication *via* the Public Engagement Training offered by *City2Science* (Herford, Germany). This training will be held in Bielefeld and will include sessions from “Science in Society” to “Responsible Research and Innovation” as well as sessions about the personal

advantages and best practice examples of science-society dialogues. Furthermore, participants will design a dialogue-oriented communication idea for their research topic, which they then can present to the lay public at science fairs like the *GENIALE* in Bielefeld or the *Long Night of Science* in Leipzig and Jena. The costs for the training itself are 2,400 € plus an estimated cost of travel (200 € × 12 participants from outside of Bielefeld) and accommodation (80 € × 12 participants × 2 nights) of 4,320 € (Total: 6,720 €).

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Conflicts of interest

The authors have declared that no competing interests exist.

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