Resources and oribatid mites: Effects on life history, chemical ecology, biochemistry and food selection

Dem Fachbereich Biologie der Technischen Universität Darmstadt
zur Erlangung des Grades eines *Doctor rerum naturalium* (Dr. rer. nat.)
genehmigte Dissertation von Adrian Brückner aus Alzenau

Tag der Einreichung: 21. März 2018,
Tag der Prüfung: 17. Mai 2018
Darmstadt 2018 — D 17

1. Gutachten: PD Dr. Michael Heethoff
2. Gutachten: Prof. Dr. Andreas Jürgens

TECHNISCHE
UNIVERSITÄT
DARMSTADT

Fachbereich Biologie
Ecological Networks

Resources and oribatid mites:
Effects on life history, chemical ecology, biochemistry and food selection

Genehmigte Dissertation von Adrian Brückner aus Alzenau

1. Gutachten: PD Dr. Michael Heethoff
2. Gutachten: Prof. Dr. Andreas Jürgens

Tag der Einreichung: 21. März 2018
Tag der Prüfung: 17. Mai 2018

Darmstadt 2018 — D 17

Die Veröffentlichung steht unter folgender Creative Commons Lizenz:
CC–BY ND 4.0 international
Namensnennung – Keine Bearbeitung 4.0 International
https://creativecommons.org/licenses/

"All boundaries are conventions, waiting to be transcended. One may transcend any convention if only one can first conceive of doing so." – **Robert Frobisher, Cloud Atlas** (by David Mitchell)

Table of Contents

1 Summary **3**

2 Zusammenfassung **5**

3 General Introduction **7**
3.1 Resources . . . 7
3.2 Oribatid mites . . . 10
3.3 Research questions . . . 13

4 Synopsis **14**

5 Conclusion **26**

6 References **30**

7 Declaration - Ehrenwörtliche Erklärung **39**

8 Acknowledgements **40**

9 Curriculum vitae **43**

10 Chapters **47**
10.1 Life history . . . 47
10.1.1 Publication 1: **Effects of nutritional quality on the reproductive biology of *Archegozetes longisetosus* (Actinotrichida, Oribatida, Trhypochthoniidae)** . . . 47
10.1.2 Publication 2: **Nutritional quality modulates intraspecific trait variability** . . . 60
10.2 Chemical ecology . . . 96
10.2.1 Publication 3: **The ontogeny of oil gland chemistry in the oribatid mite *Archegozetes longisetosus* Aoki (Oribatida, Trhypochthoniidae)** . . . 96
10.2.2 Publication 4: **Nutritional effects on chemical defense alter predator-prey dynamics** . . . 103
10.3 Biochemistry . . . 113
10.3.1 Publication 5: **Biomarker function and nutritional stoichiometry of neutral lipid fatty acids and amino acids in oribatid mites** . . . 113
10.3.2 Publication 6: ***De novo* biosynthesis of simple aromatic compounds by an arthropod (*Archegozetes longisetosus*)** . . . 123
10.4 Food selection . . . 145
10.4.1 Publication 7: **Track the snack – olfactory cues shape foraging behaviour of decomposing soil mites (Oribatida)** . . . 145
10.4.2 Publication 8: **Imprinted or innate food preferences in the model mite *Archegozetes longisetosus* (Actinotrichida, Oribatida, Trhypochthoniidae)** . . . 153
10.5 Appendix . . . 158
10.5.1 Supplementary information - Publication 2 . . . 158
10.5.2 Supplementary information - Publication 4 . . . 167
10.5.3 Supplementary information - Publication 6 . . . 171
10.5.4 Supplementary information - Publication 7 . . . 176

1 Summary

Nutrition affects nearly all traits and physiological process of an organism and especially heterotrophs are regulated by nutrient quality. Numerous studies have demonstrated the influence of nourishment on herbivorous insects, but also on "classical" model organisms (e.g. the fruit fly or mice). The relationship of nutrients, trait expression and food selection, in detritivores, however, remains uncertain. For this thesis I hence focused on the influence of resources on multiple traits of oribatid mites (life history, chemical ecology and biochemistry). Oribatids are a speciose group of soil-dwelling micro-invertebrates which are globally present in virtually all terrestrial habitats, fostering soil structure and fertility by contribution to elemental cycles. I was specifically interested in how defensive as well as life-history traits and the flow/incorporation of biomolecules from resources into animals, are affected by diets and which mechanisms underlie resource selection. To elucidate these relationships, I used the parthenogenetic oribatid mite *Archegozetes longiestosus*, which has been used as a model organisms for more than 25 years. This mite possesses many, easily measurable life-history and morphological traits. Furthermore, it has large opisthonotal glands, which synthesize chemical defensive secretions used as strong repellent agents against predators, thus influencing the structure and stability of soil food webs. In general, I could demonstrate that life history parameters of *A. longisetosus* were affected by resources and that the foods' elemental stoichiometry could partly explain these patterns. Furthermore, nutritional quality did not only influence the mean expression of traits, but also their variation. Consequently, I found a stoichiometric optimum for trait variation – i.e. Bertrand's and threshold-elemental-ratio rule like relationships. Focusing on chemical ecology, age (ontogeny) as well as nutritional supplementation influenced the relative composition and amount of the mites' defensive secretions. I used this data to parametrize a functional response model for chemical defense that revealed a strong nutritional correlation of long-term chemical defense and nutrients (especially sugars and fats). Regarding the translocation of specific biomolecules, I demonstrated a direct trophic transfer of neutral lipid fatty acids from the food to mites, rendering fatty acids as reliable biomarkers for food web ecology. In contrast, amino acids were only selectively utilized, leading to a homeostasis of amino acid composition in mites. My results also indicate that all, but especially the highly bioactive aromatic compounds from the opisthonotal glands are synthesized *de novo* from carbohydrate/fat derived precursors, once again highligh-

ting the importance of sugars and fats for the maintenance of an effective chemical defense. Olfactory preference tests with semi-natural resources (bacteria, fungi, lichen and litter powder) – emitting different volatile organic compounds and differing in their resource composition – revealed an innate olfactory preference of oribatid mites. *Archegozetes longiestosus* especially favored food which was rich in fatty acids, suggesting a potentially adaptive connection of resource selection and chemical anti-predator defense – thus spanning two trophic levels – in oribatid mites.

Keywords: nutritional ecology, evolutionary ecophysiology, soil food web, mites, biochemical ecology

2 Zusammenfassung

Nahrung wirkt sich entscheidend auf nahezu alle Eigenschaften und physiologischen Prozesse von Organismen aus. Insbesondere Heterotrophe scheinen dabei besonders durch die Nahrungsqualität reguliert zu sein. Während zahlreiche Studien zum Einfluss von Nahrung bei herbivoren Insekten, sowie „klassischen" Modellorganismen (bspw. Taufliegen oder Mäuse) existieren, ist nur wenig über die Zusammenhänge von Nährstoffen und Fitness, sowie Performanz bei Zersetztern (= Detritivore) bekannt. Im Rahmen dieser Arbeit habe ich mich daher mit den Einflüssen von Nahrung auf zahlreiche Eigenschaften von Hornmilben befasst. Bei Hornmilben (auch Moosmilben) handelt es sich um eine artenreiche Gruppe von bodenbewohnenden Kleinstwirbellosen („Mikroinvertebraten"), die weltweiten in nahezu allen terrestrischen Habitaten vorkommen und die Ausbildung von Bodenstrukturen, sowie die Bodengesundheit/-fruchtbarkeit fördern, und an Energie- und Nährstoffkreisläufen beteiligt sind. Dabei interessierte mich besonders, wie Nahrung die Verteidigung und Lebenslauf-Eigenschaften („life history traits"), sowie die Stoffflüsse und den Einbau von Biomolekülen aus der Nahrung ins Tier beeinflusst und welche Mechanismen der Nahrungswahl zugrunde liegen. Zur Klärung dieser Zusammenhänge habe ich die parthenogenetische Hornmilbenart *Archegozetes longiestosus* verwendet, die sich innerhalb der letzten 25 Jahre als eine Musterspezies zur Erforschung von Hornmilben, sowie bodenbiologischen Fragestellungen etabliert hat. Diese Milbe besitzt viele einfach zumessende Lebenslauf-Eigenschaften und morphologische Eigenschaften. Darüber hinaus besitzt sie große opisthontale Drüsen, die eine Mischung von chemischem Verteidigungssekret synthetisieren, das als effektives Repellent gegen Fraßfeinde angewendet wird. Dementsprechend beeinflusst chemische Verteidigung die Struktur, sowie die Stabilität von Bodennahrungsnetzen. Generell wurden alle Lebenslauf-Eigenschaften von *A. longiestosus* durch Ressourcen beeinflusst und die stöchiometrische Zusammensetzung des Futters kann diese Änderungen zumindest teilweise erklären bzw. vorhersagen. Weiterhin zeigte sich, dass Nahrungsqualität nicht nur den Mittelwert einer bestimmten Eigenschaft („trait") beeinflusst, sondern auch dessen Variation. Dementsprechend, habe ich stöchiometrische Optima für die Eigenschaftsvariation gefunden; ein Zusammenhang, der auch als „Betrand's Regel" oder „Grenzwert-Elementeverhältnis" bekannt ist. Fokussierend auf die chemische Ökologie der Musterspezies fand ich, dass sowohl Alter („Ontogenie") als auch Zusammensetzung bereitgestellter Nährstoffe einen Einfluss auf die

Komposition, sowie die Menge des Verteidigungssekretes hat. Diese Daten habe ich zur Parametrisierung eines Modelles, das die funktionelle Reaktionsantwort von chemisch beeinflussten Räuber-Beute-Beziehung über die Zeit beschreibt verwendet. Dieses numerisch-ökologische Modell zeigte, dass die Aufrechterhaltung von chemischer Verteidigung über längere Zeiträume mit den Nährstoffen in der Nahrung, insbesondere Kohlenhydraten und Fetten, zusammenhängt. In Bezug auf die Translokation von spezifischen Biomolekülen konnte ich einen direkten trophischen Transfer von Fettsäuren neutraler Lipide von der Nahrung in das Fettgewebe der Milben nachweisen. Dies bestätigte den Einbau von Fettsäuren als verlässliche Biomarker in der Nahrungsnetzökologie. Im Gegensatz dazu fand ich eine selektive Verwendung von Aminosäuren, die eine Homöostase dieser Stoffklasse für Hornmilben nach sich zieht. Darüber hinaus konnte ich ebenfalls beweisen, dass die hoch bioaktiven Aromaten im Wehrsekret von *A. longiestosus* de novo aus kohlenhydrat- oder fett-abgeleiteten Vorläufermolekülen synthetisiert werden können. Diese belegte nochmal die Wichtigkeit von Zuckern und Fetten zur Aufrechterhaltung einer effektiven chemischen Verteidigung. Olfaktorische Wahlversuche mit semi-natürlichen Ressourcen (Bakterien-, Pilz-, Flechten- und Laubstreupulver), die verschiedene flüchtige organische Substanzen emittierten und eine unterschiedliche Nährstoffkomposition aufwiesen, offenbarten eine immanente olfaktorische Präferenz bei Hornmilben. *Archegozetes longiestosus* bevorzugte dabei insbesondere Futter mit einem hohen Anteil an Fettsäuren. Dies legt den Schluss einer möglichen adaptiven Beziehung von Ressourcenselektion und chemischer Verteidigung gegen Fraßfeinde über zwei trophische Ebenen für Hornmilben nahe.

Schlagworte: Ernährungsökologie, evolutionäre Ökophysiologie, Bodennahrungsnetz, Milben, biochemische Ökologie

3 General Introduction

3.1 Resources

The diverse, multidimensional and often times enigmatic structures of and dynamics within ecological systems are largely a consequence of the distribution, availability and partition of resources (Brown et al., 2004; Lennox and Wilson, 1994). Organisms consume resources from their habitats, ingest, process and transform them into energy and metabolic products via biochemical reactions, use these products to enhance their fitness and finally excrete metabolized materials back into the environment (Simpson and Raubenheimer, 2012; Sperfeld et al., 2016; Sterner and Elser, 2002). The translocation of resources across food webs and ecosystems is therefore the universal interconnection, but also the currency in the web of life. Consequently, nutrition can help to explain the bases of ecosystem level effects by tracing them to the level of their ultimate cause – the physiology and biochemistry of individuals (Elser et al., 1996).

While nutrients certainly connect all living things in an ecosystem, but also on an individual level, nutrition itself is also complex and can be conceptualized in many ways (Simpson and Raubenheimer, 2012). For instance, food resources can be described as multicomponent blends of macronutrients (carbohydrates, fat and protein; **Figure 1a**) and micronutrients (minerals, sterols, vitamins) or as mixtures with a specific elemental ("stoichiometric") composition (**Figure 1b**). The first concept outlines the complex nature of nutrients, but fails to separate cofounding effects between nutrients. While the latter oversimplifies the chemical nature of biomolecules to elemental ratios, it has a high predictive potential across systems (Elser, 2003; Kay et al., 2005; Sterner and Elser, 2002). Both concepts, however, do not account for the fact that nutrients and elements of individual foods cannot be consumed singularly because they are mixtures of distinct composition (Simpson and Raubenheimer, 2012; Sperfeld et al., 2016). Raubenheimer and Simpson (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993) accounted for this problem - the ingestion of nutrient y necessarily leads to the ingestion of nutrient z - in their Geometric Framework of Nutrition (GF) by proposing such called "nutritional rails" (**Figure 1c**). Using the GF it became clear that most animals cannot survive feeding on a one dimensional resource (i.e. consumption along one nutritional rail), but need different foods (i.e. feeding across the nutritional rails) to reach a point of nutritional balance result-

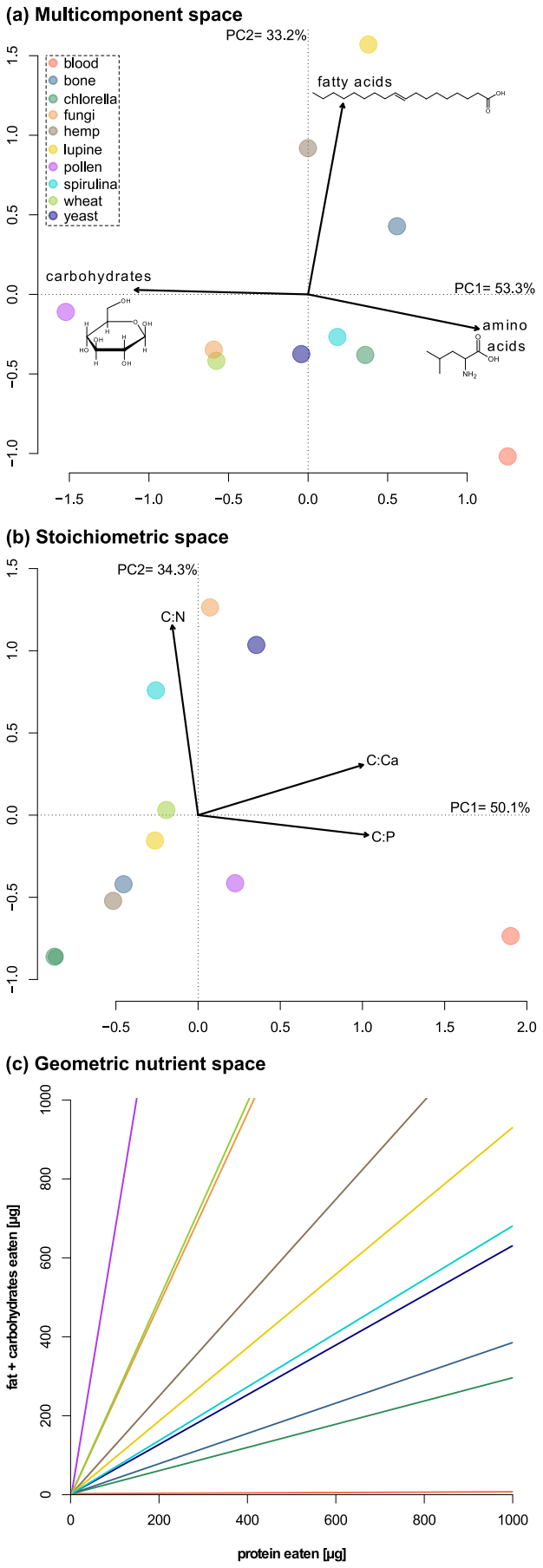


Figure 1 – Different concepts to visualize the complex nature of nutrition and nutrients. (a) Principal component analysis (PCA) biplot of the macronutrient content of ten different resources which are used throughout this thesis. (b) PCA biplot of the elemental ratio composition constituting a stoichiometry space for this resources. (c) Protein-carbohydrate/fat nutrient space for the resources; the differently colored radials represent the nutritional balance of the nutrients in each food ("nutritional rails"; sensu Raubenheimer and Simpson, 1993). Colors correspond to the figure legend in (a), arrows represent the amino acid, fatty acid and carbohydrate amount (in a) or the elemental C:Ca, C:N and C:P ratios (in b) expressed as loadings.

ing in maximized Darwinian fitness (Simpson and Raubenheimer, 2012). This species-specific optimum is called the intake target (Raubenheimer and Simpson, 1993; 1997).

While the intake target appears to be a good predictor of an animal's response to a certain food source in terms of fitness (Raubenheimer and Simpson, 1997; Simpson and Raubenheimer, 2012), the ultimate reason why it responds is much harder to explain. This is because, even at the intake target, food is still a complex blend and its compounds can have different functions in an animal's metabolism (Raubenheimer and Simpson, 2009). While one food component can be beneficial for the optimal expression of one trait, it can be disadvantageous for another one (**Figure 2**; "trade-off"; Raubenheimer and Simpson, 2009; Stearns, 1989). From an animal´s perspective, its traits depend on multiple nutritional factors and not singularly on a particular compound. Finally, food compounds are interconnected via biochemical pathways and also in their effect on an animal. Yet, in essence, resources are of pivotal significance for all vital physiological process within a single individual (Simpson and Raubenheimer, 2012). No single natural food can, however, offer a perfectly balanced nourishment and hence trade-offs must exist (**Figure 2**; Stearns, 1989). While one food can cause a beneficial change in one trait, this positive effect is linked to a detrimental change in another character. If there were no nutritional based trade-off (over an evolutionary time scale) most organisms would feed on very similar composed resources which drive all traits towards a fitness maximum (Stearns, 1989; 2000). Hence, nutritional limitations form the basis for life-history trade-offs (**Figure 2**; Stearns, 1989; 2000; Raubenheimer and Simpson, 2012). In general, trade-offs exist on the phenotypic and genotypic level (Sterans, 1989). Natural selection, however, acts on the phenotype (ultimately linked to the variation of the genotype) and thus phenotypic trade-offs drive the response of observable life-history effects towards a certain (nutritional) gradient (Schlichting and Pigliucci, 1998; Stearns, 1989). The classical view considers the allocation of resources as the main factor of phenotypic life-history trade-offs: more food results in more available energy that can be invested to enhance fitness and hence the availability of enough food eliminates trade-offs (Boutin, 1990; Houle, 1991; Van Noordwijk and de Jong, 1986; see also Lailvaux and Husak, 2014). In contrast recent studies (Jensen et al., 2011; Mayntz et al., 2005; Simpson et al., 2004; Vanni and Lampert, 1992) showed that quality (i.e. balanced macronutrients and/or elements) rather than the amount of allocatable food has profound effects on an animal's life history. In fact, some animals adjust the amount of ingested food to deal with imbalanced diets

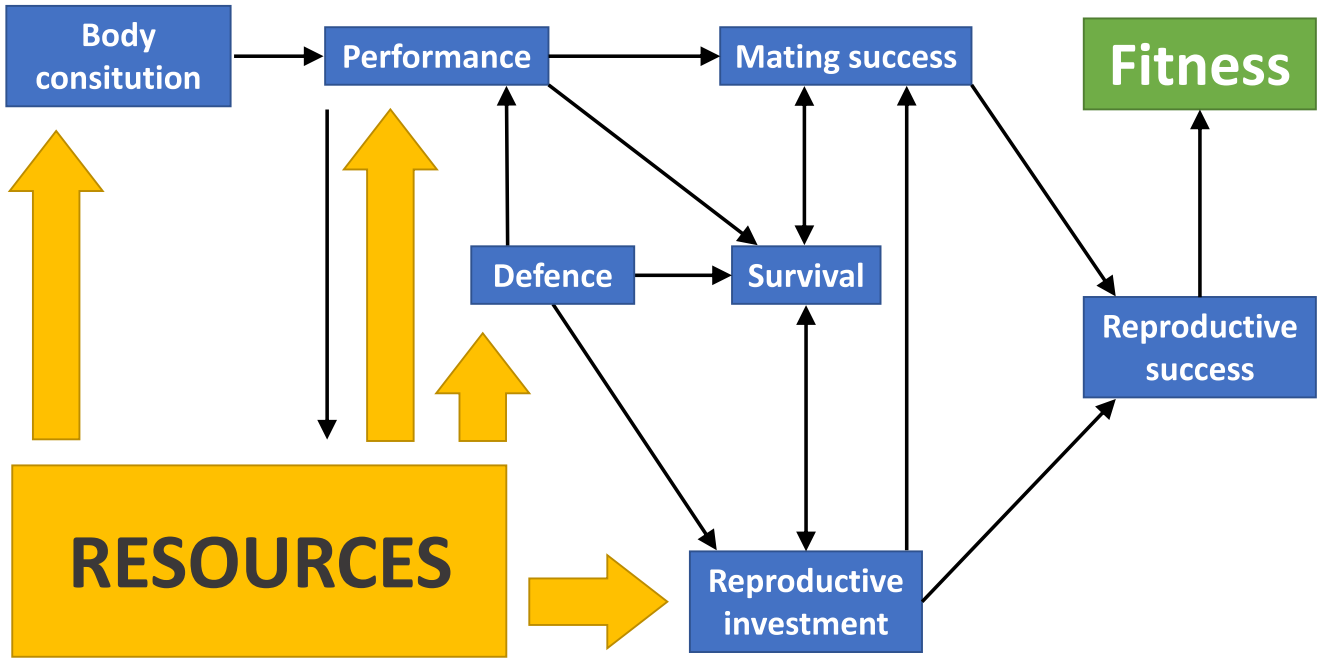


Figure 2 – The relationship of traits and resources in a hypothetic species (adapted from Lailvaux and Husak, 2014; see also for detailed outline). This general framework illustrates the complex connection of traits, resources quality and potential trade-offs. While some of the links have strong empirical support from many species, the existence of some others is questionable and also in no case have all resource-to-trait associations been conclusively tested within a single species. "Defense" might be any strategy which enhances a species' survival (immunity, chemical or morphological anti-predator defense). "Body constitution" means a species' morphology and elemental as well as macronutrient stoichiometry. Broad arrows represent a direct influence of resources on a certain character, while thin black arrows denote an effect of character y on character z.

by either restricting their eating or by overeating a resource to acquire the nutrients or elements in short supply necessary to reach their intake target (**Figure 1c**; Raubenheimer et al., 2007; Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 2012; Sørensen et al., 2008).

3.2 Oribatid mites

Oribatid mites (Arachnida, Actinotrichida, Oribatida) are a speciose arthropod group comprising 16,000 described species, including Astigmata (Schatz et al., 2011). The "traditional" oribatid mites (~11,000 species) are ubiquitously, but unevenly distributed across different microhabitats (e.g., dead wood, lichens, litter, moss, mineral soil and tree bark) of all terrestrial ecosystems around the world (Maraun et al., 2007; Wehner et al., 2016) and can reach abundances of hundreds of thousands of individuals per square meter (Maraun and Scheu, 2000). Oribatid

mites are an evolutionary very old group of organisms and were among the first animals invading terrestrial habitats in the Devonian age, constituting the first, simple soil food webs (Norton et al., 1988; Schaefer et al., 2010; Shear et al., 1984). Focusing on nutrition preferences, oribatid mites can be classified as lichen feeders, primary as well as secondary decomposers and predators/scavengers (Heidemann et al., 2011; Maraun et al., 2011; Schneider et al., 2004). Whereas primary decomposers mainly feed on dead plant material (mostly of poor nutritional quality), secondary decomposers consume a variety of more nutritious food, for instance different species of algae, bacteria and fungi (Luxton, 1972; Norton, 2007; Schuster, 1956; Schuster, 1979).

As for the general quantity/quality nutrient debate in life-history evolution, it appears that many terrestrial decomposers (which basically live in their food) are not bottom-up controlled by means of resources availability, but rather by the quality as well as patchy distribution of high-quality nourishment (Booth and Anderson, 1979; Jørgensen et al., 2008; Van Amelsvoort and Usher, 1989). Thus, access to high quality food is supposed to be a limiting factor of oribatid mites' fitness as well (Norton, 1994). Since oribatids have low metabolic rates (Ehnes et al., 2014; Luxton, 1975; Thomas, 1979) and may be unable to store large amounts of energy for a long time (Wallwork, 1979), compensatory feeding (restricting or overeating) to reach an intake target or to allocate resources in short supply for further storage, seems unlikely. Hence, the input of more nutritious high-quality food should evoke a relatively quick response, which directly loops back to all levels of oribatid mite life history (see also Norton, 1994). Yet, these interconnections are largely unstudied in oribatid mites (Norton, 2007; Smrž, 2010). One way to entangle these connections, is to use nutritionally stressful conditions (i.e. *ad libitum* excess to one imbalanced food or restricted amounts of balanced food) to challenge animals to trade-off among traits, thereby revealing which life-history and performance parameters are correlated to each other via shared nutritional demands (Jensen et al., 2011; Zera and Harshman, 2001).

I adapted this strategy and used the parthenogenetic, quasi-clonal oribatid mite *Archegozetes longisetosus* Aoki 1965 (Heethoff et al., 2013) as a focal model species in all studies of this thesis. Other than sexual species, where heritable genetic variation can hardly be distinguished from phenotypic trait variation, clonal systems provide the possibility to quantify phenotypic responses of traits (Norton, 1994; Stearns, 1989; Woltereck, 1909). This renders *A. longisetosus* as a "phenotypically tractable" model species to study the multidimensional effects of

nutrition on phenotypic traits and trade-offs. One major performance trait of oribatid mites, investigated in this thesis, relates to their defensive exocrine opisthonotal oil-glands (Brückner et al., 2017; Heethoff et al., 2011; Raspotnig, 2010) – a synapomorphic character of all "glandulate" oribatid mites (Raspotnig, 2010). The biological role of these opisthonotal glands in "traditional" oribatid mites was rather speculative for a long time. Some authors proposed lubricating and osmo- or thermoregulative function (Riha, 1951; Smrž, 1992; Zachvatkin, 1941), yet others assumed a major role in chemical communication based on evidence from Astigmata where more than 30 chemical compounds have been identified as alarm-, aggregation-, or sex-pheromones (Kuwahara, 2004). Focusing on "glandulate" oribatid mites (i.e., Parhyposomata, Mixonomata, Desmonomata and Brachypylina) about ∼130 compounds (Raspotnig et al., 2011) have been identified. While some appear to be alarm pheromones (Raspotnig, 2006; Shimano et al., 2002), most function as defensive allomones against predators in adult and juvenile oribatid mites (Heethoff et al., 2011; Heethoff and Raspotnig, 2012a). Reservoir based defensive chemistry plays an important role in structuring and stabilizing feeding interactions in soil food webs and enables the oribatid mite to live in an "enemy-free space" (e.g., Brückner et al., 2016; Heethoff et al., 2011; Heethoff and Rall, 2015; Peschel et al., 2006). Interestingly, some of these compounds – the alkaloids – were shown to be the ultimate source of most toxins sequestered by poison-frogs (Saporito et al., 2007; Saporito et al., 2011).

3.3 Research questions

My main goal with this thesis was to unravel the complex relationships of nutrients in a "phenotypically tractable" oribatid mite model species. Based on the above outlined frameworks for nutrition (**Figure 1**) and trait interconnection (**Figure 2**), I asked the following questions:

Q 1: How does food quality (and stoichiometric balance) affect the reproductive biology, (functional) traits and performance of *Archegozetes longisetosus* on a phenotypic level and are there trade-offs among different traits?

Q 2: How does macronutrient quality changes the defensive gland chemistry of *Archegozetes longisetosus* and are there functional consequence for predator-prey interactions?

Q 3: Is there a translocation of specific food nutrients used for the biosynthesis of defensive compounds and how are major macronutrient classes (fat and protein) stoichoimetrically regulated in *Archegozetes longisetosus*?

Q 4: Are oribatid mites able to locate and discriminate among food resources and if so which sensory mechanism might be responsible?

4 Synopsis

Publication 1: **Effects of nutritional quality on the reproductive biology of Archegozetes longisetosus (Actinotrichida, Oribatida, Trhypochthoniidae)**

Adrian Brückner, Romina Schuster, Katja Wehner and Michael Heethoff (2018)

Soil Organisms 90: 1-12

The focal model organism of this thesis – the parthenogenetic trhypochthoniid oribatid mite *Archegozetes longisetosus* – is certainly the best studied oribatid mite (Heethoff et al., 2013). Yet, published life history studies, which also included the effects of food are conflicting. Some studies claim that nutrition has no considerable effects on *A. longisetosus* at all (Estrada-Venegas et al., 1999; Haq and Adolph, 1981; Haq and Prabhoo, 1977), while others could show an influence on the number of offspring, developmental time, mortality and morphology (Seniczak, 1998; 2006; Seniczak et al., 2016; Smrž and Norton, 2004). In this paper (**publication 1**) we aimed to clarify how nutrition affects the life history parameters. To do so, we set up a large scale experiment with ten different food resources of various origins and elemental composition (animal, bacterial, fungal and herbal; see also **Figure 1**) and observed the mites' development for about three months. Additionally, we performed an elemental analysis (C/N, C/P and C/Ca-ratio) of the food to describe its nutritional value. We generally found that food affected all life history parameters of *A. longisetosus*, which could partly be explained with the foods' elemental stoichiometry. For instance, the number of offspring ranged from 0 to 106 individuals per female, while the developmental time and body mass varied in a range of 32 up to 88 days and 3 to 43 μg dry weight, respectively. The number of offspring per female was correlated to the C/N-ratio, while the body mass of early juveniles was correlated to the C/P- and C/Ca-ratio. The developmental time of the different instars was not correlated to any nutritional parameter. Since trade-offs among certain life-history traits are quite common in invertebrates (Nunney, 1996; Olofsson et al., 2009; Simpson and Raubenheimer, 2012; Zera and Harshman, 2001), we also tested if there is one between the number and developmental time of offspring. This was, however, not the case for *A. longisetosus* and in contrast, we found that some particularly

balanced food resources are able to sustain both; high reproduction and shorter developmental time. These results overall suggest that there is a relatively distinct nutritional spectrum (or intake target; Raubenheimer and Simpson, 1993; 1997) for *A. longisetosus* and also an optimum response of traits towards a nutritional gradient ("Bertrand's rule" or "threshold elemental ratio, but see **publication 2**).

Publication 2: **Nutritional quality modulates intraspecific trait variability**

Adrian Brückner, Romina Schuster, Katja Wehner and Michael Heethoff

Submitted to Frontiers in Zoology

While the former publication (**publication 1**) distinctly focused on the reproductive biology and the effects of nutrient on the mean trait value, we explored the effects of nutritional quality on a much broader set of traits from different classes (chemical defense, life history, morphometric parameters) and their intraspecific variation in this study (**publication 2**). Trait based functional and community ecology is *en vogue* (Krause et al., 2014; Violle et al., 2007). Since most studies only consider the trait mean, but not trait variation, the fact that natural populations consist of phenotypically diverse animals is often neglected (Bolnick et al., 2011; Whitman and Agrawal, 2009). As mentioned before (**general introduction**), theory as well as experimental evidence states that variation of traits is ubiquitous (Schlichting and Pigliucci, 1998; Whitman and Agrawal, 2009) and may arise (besides several other mechanisms) from elemental and nutritional imbalances of food resources (Simpson and Raubenheimer, 2012; Sterner and Elser, 2002). Since conclusive experiments, which demonstrate how a set of traits of one species can vary (i.e. intrinsic potential of trait variation) are lacking, we again harnessed the phenotypic tractability of *A. longisetosus* (see also **general introduction** and **publication 1**, **5**). Specifically, we aimed to decipher patterns of dietary induced trait plasticity and quantify the means as well as intra- and inter-trait variation along a nutritional gradient (**Figure 1**). We could demonstrate that nutritional quality (measured as C/N ratio) influenced i) all trait means and ii) their variability. More importantly, the (eco)-physiological paradigm known as "threshold elemental ratio" (for element stoichiometry; Boersma and Elser, 2006) or as "Bertrand's rule" for marcro-/micronutrients (Bertrand, 1912; Raubenheimer et al., 2005) not only applies to trait means, but also to trait variation. In consequence, this suggests that a nutritionally or elementally balanced nutrition leads to a high mean trait value and a low variation. Based on our experimental data derived from a quasi-clonal model system, we hence proposed that *mean trait variation* might be predictive for the food quality, while the *variation of trait variation*

(across many trait of animals fed on one resources) may be an indicator for trade-offs an animal has to deal with while feeding on a particular nourishment.

Publication 3: **The ontogeny of oil gland chemistry in the oribatid mite Archegozetes longisetosus Aoki (Oribatida, Trhypochthoniidae)**

Adrian Brückner and Michael Heethoff (2017)

International Journal of Acarology 43: 337-342

De novo produced defensive allelochemicals are affected by various factors which influence the quality and quantity of glandular exudates. For instance, age (Blum, 1996; Bodner and Raspotnig, 2012), sex (Kullenberg et al., 1970; Unruh et al., 1998; Whitman et al., 1992) and especially diet (Holliday et al., 2009; Jones et al., 1987) can influence the degree of being chemically defended. Thus, as a first step before exploring the effects of diet and specific nutrients on the defensive gland secretions of *A. longisetosus* (**publication 4** and **6**), we investigated whether the species' oil gland chemistry is altered during ontogeny. In *A. longiseotus*, the oil gland reservoirs and their contents remain with shed exuviae, hence the secretions need to be resynthesized after each molt. Consequently, we analyzed the complete ontogenetic sequence (larva, protonymph, deutonymph, tritonymph and adult) of oil gland secretions using gas chromatography – mass spectrometry. The absolute and body mass-corrected amounts of oil gland exudates increased during ontogeny, yet this trend was not strictly linear, but scaled allometrically with the body mass of ontogenetic instars. As expected from studies on other arthropods (Blum, 1996; Bodner and Raspotnig, 2012), we found ontogenetic shifts in the relative composition of defensive chemicals between instars, while the qualitative composition (= 2,6-HMBD, neral, neryl formate, γ-acaridial, tridecane, 7-pentadecene, pentadecane, 6,9-heptadecadiene, 8-heptadecene and heptadecane) was stable. Overall, these results indicated a potential metabolic energy trade-off between growth and chemical protection in juvenile oribatid mites. Yet, the main purpose of this paper (**publication 3**) was the establishment of suitable methods, to study the effects of nutrients on chemical defense (**publication 4**) and of specific precursor translocation (**publication 6**).

Publication 4: **Nutritional effects on chemical defense alter predator-prey dynamics**

Adrian Brückner and Michael Heethoff (2018)

Chemoecology 28: 1-9

As mentioned before (**publication 3**) many factors can affect the defensive chemistry of an organism. For example, unbalanced diets are a major cause of physiological stress, which also appears to influence autogenously defended animals (Jones et al., 1986; 1987; 1989). Thus, we again used our ten-resource treatment (**Figure 1; publication 1**) to study their effect on chemical defense. To also shed light on the possible consequences of this alterations and derive testable hypothesis about the connection of nutrition and food web stability, we used an experimentally derived numerical predator-prey model (Heethoff and Rall, 2015). This model conceptualizes reservoir-based chemical defense, which is a widespread mechanism to repel predators in many invertebrates, as a functional response model (= reducible defense). We measured the oil gland exudates of *A. longisetosus* after artificial gland depletion (Heethoff and Raspotnig, 2012b) to estimate the regeneration dynamics (Heethoff, 2012) for each resource and parameterized the theoretical functional response model with these data. Regeneration rates were correlated to the amount of dietary fat and carbohydrates (i.e. available energy in the food). Furthermore, the modeling approach revealed that higher regeneration rates are particularly beneficial under permanent predation pressure (as assumed in our model). We hence derived the "*energy-regeneration rate hypothesis*" (i.e. food with higher caloric value yield higher regeneration; see also **publication 6**) and the "*regeneration-effective chemical protection hypothesis*" (i.e. higher regeneration results in a more stable long-term chemical protection) which lead themselves to further experimental testing. This again underpins the pivotal need to integrate experimental chemical and theoretical predator-prey ecology to better predict the interactions and stability of (soil) food webs.

Publication 5: **Biomarker function and nutritional stoichiometry of neutral lipid fatty acids and amino acids in oribatid mites**

Adrian Brückner, Andrea Hilpert and Michael Heethoff (2017)

Soil Biology and Biochemistry 115: 35-43

While we focused on broad scale effects of bulk nutrients or elements in the previous studies (**publication 1**, **2** and **4**), we specifically focus on specific biomolecules in this (**publication 5**) and two further studies (**publication 6** and **7**). Among many different methods to unravel complex terrestrial (soil) food webs (Birkhofer et al., 2017), biomarkers like fatty acids, amino acids, stable isotopes, or molecular barcodes have become increasingly important to study the translocation of matter and nutrients across resources and consumers (Birkhofer et al., 2017; Ruess et al., 2002). While this biomarker function of fatty acids has been widely investigated for some animal taxa (e.g., ants, collembolans and nematodes, see Rosumek et al., 2017; Ruess and Chamberlain, 2010), the potential for characterizing soil-dwelling oribatid mites remained unknown. Hence, we again used the power of our "phenotypically tractable" model species and the "ten resources" approach (**Figure 1; publication 1**) to characterize the translocation of fatty and amino acids between *A. longisetosus* and its food as well as the biomarker potential. Therefore, we analyzed the neutral lipid fatty acids (NLFA) and amino acids of the opportunistic model oribatid mite species and the ten different resources of animal, bacterial, fungal and herbal origin with gas chromatography/mass spectrometry (GC/MS) and ion-exchange chromatography (IEC). We found diet-specific amounts and a correlated composition of resource and oribatid mite NLFAs. Amino acids of mites, however, were stable and independent of diet. We could further assign the consumer NLFA composition to distinct feeding groups, rendering fatty acids as reliable biomarkers for oribatid mites. The amino acid profiles appear to reflect diet-independent intrinsic physiological properties and confirm the *homeostatic protein stoichiometry hypothesis* (Anderson et al., 2004) for oribatid mites.

Publication 6: **De novo biosynthesis of simple aromatic compounds by an arthropod (*Archegozetes longisetosus*)**

Adrian Brückner, Martin Kaltenpoth and Michael Heethoff

Submitted to Proceedings of the National Academy of Sciences of the United States of America

Similar to our study about amino and fatty acids (**publication 5**), we were again interested in the translocation of specific nutrients, yet this time we focused on potential precursor molecules for chemical defense. The defensive secretion of *A. longisetosus* consists of ten different chemicals, namely: 2,6-HMBD, neral, neryl formate, γ-acaridial, tridecane, 7-pentadecene, pentadecane, 6,9-heptadecadiene, 8-heptadecene and heptadecane (see also **publication 3**). The biosynthetic routes leading to the terpenes (mevalonate pathway) and hydrocarbons (fatty acid pathway) are straight forward (Morgan, 2010; Walsh and Tang, 2017). The biosynthesis of the two aromatics remains, however, enigmatic. This is because arthropods seem to lack this ability to synthesize benzenoids *de novo* (Morgan, 2010; Pankewitz and Hilker, 2008), and hence rely on aromatic amino acids acquired from food or on symbiotic microorganisms (Hertweck, 2009; Morgan, 2010). The latter together with fungi and plants share an exclusive biosynthetic route leading to aromatic amino acids – the shikimic acid pathway (Walsh and Tang, 2017). Some organisms (including the former, but also some protozoans and some vertebrates) further evolved the polyketide pathway as an additional biochemical route to form core benzenoids (Hertweck, 2009). Here aromatics are synthesized via a head-to-tail condensation of β-polycarbonyls followed by a cyclization (Birch and Donovan, 1953). A few studies on arthropods (e.g., ants and harvestman), indicated that this *de novo* biosynthesis might also occur, but potential endosymbiotic bacteria were never excluded. To clarify these inconclusive results, we again used the defensive secretions of *A. longisetosus* to unravel the biosynthetic logic of aromatic compound biosynthesis in arthropods. Therefore, we excluded endosymbiotic bacteria using intensive antiobiotic treatment (10% oral) and additionally supplemented the mites with a variety of stable-isotope labeled precursor molecules. The incorporation of the heavy isotopes was monitored with mass spectrometry, showing that *A. longisetosus* can autogenously synthesize both simple benzenoids, probably via the polyketide pathway. This study did not only

show that arthropods can synthesize aromatics *de novo*, but also supported that the model mite is able to produce its defensive compounds only from carbohydrate derived compounds, again underpinning the results of our modeling study (**publication 4**).

Publication 7: **Track the snack – olfactory cues shape foraging behaviour of decomposing soil mites (Oribatida)**

Adrian Brückner, Romina Schuster, Timo Smit, Melanie M. Pollierer, Irmgard Schäffler and Michael Heethoff (2018)

Pedobiologia 66: 74-80

Experimental evidence (gathered from our former studies) suggested that resources which are nutritionally "good" for biosynthesizing glandular secretions are not necessarily "good" for essential life-history parameters like reproductive output or developmental time (**publication 2** and **4**). Consequently, oribatid mites should either possess olfactory or gustatory based sensory mechanisms (De Lillo et al., 2004; Raspotnig, 2006) to find and allocate "optimal" resources (i.e. food that meets their current or general nutrient requirements). Further, on numerous occasions (especially while performing the experiments for **publication 1** and **2**) we observed that mites, even if they are blind, appear to "quickly" head towards a food source. Hence, we hypothesized that some kinds of olfactory senses should be involved in this foraging behaviour. Since the olfactory sense heavily depends on a species' food preference (and presumably its specialization), we explored this problem with two model species: the well-known opportunistic feeder *A. longisetosus* as well as a myco-/phytophagous feeding *Scheloribates* species. We used laboratory choice bioassays with both species and different semi-artificial foods (freeze-dried and grinded bacteria, fungi, lichen and litter powders) to test our hypothesis. Generally, we found that oribatid mites use olfactory cues to find and differentiate among resources while foraging. Interestingly, we found differences in olfactory cues response between the species (*A. longisetosus* preferred bacterial diet, while *Scheloribates* favored fungi powder) which could be explained by their different feeding habits. In a manipulative experiment (with modified food powders), both species responded to semi-volatiles which were typical for their diet choice; *A. longisetosus* preferred fatty acids (often times signals for bacterial activity), while *Scheloribates* was attracted by the mushroom-alcohol 1-octen-3-ol (a universal volatile of damaged fungal tissue). Thus, we proposed the hypothesis that generalist mites use rather unspecific signals, whereas more specialized feeders react to more specific semi-volatiles to localize and discrimi-

nate their food. Interestingly, fatty acids appear to be key substances for food localization in *A. longisetosus*, suggesting an adaptive connection of food choice, defensive compound synthesis and long-term chemical defense (see also **publication 4** and **6**).

Publication 8: **Imprinted or innate food preferences in the model mite Archegozetes longisetosus (Actinotrichida, Oribatida, Trhypochthoniidae)**

Adrian Brückner, Romina Schuster, Timo Smit, and Michael Heethoff (2018)

Soil Organisms 90: 23-26

The results of the former study (**publication 7**), but also the fact that oribatid mites prefer to consume certain dark pigmented fungi (Schneider et al., 2004) directly led us to the question, if opportunistic oribatid mites have an innate or learned preference for food. To test this we used the same setup as in the former experiment (**publication 7**) and four different *A. longisetosus* strains (namely: chlorella, lupine, wheat and yeast) taken from the "ten resources"-experiments (**publication 1**, **2**, **4** and **5**). We found that *A. longisetosus* did not prefer food it has fed on before ("imprinting"), but rather favored high quality food ("innate"). These preferences were again linked to the fatty acids in the food, underpinning our finding from **publication 7**.

5 Conclusion

The paradigm of nutritional biology states that food quality basically affects all traits and interconnection of traits within an organism (Simpson and Raubenheimer, 2012; Sterner and Elser, 2002). While the overall effect of nutrients on life history and performance has strong empirical support from many species (e.g., Lailvaux and Husak, 2014; Raubenheimer and Simpson, 1993; 1997; Simpson and Raubenheimer, 1993; 2000; Simpson et al., 2004; Vanni and Lampert, 1992), there was a lack of conclusively tested evidence for trait-trait as well as resource-trait associations on the phenotypic level within a single species (**Figure 2**). I was able to find and elucidate some of these links and disentangle how resource quality is connected to life history (**publication 1** and **2**), chemical ecology (**publication 4**), biochemistry (**publication 5** and **6**) and food choice (**publication 7** and **8**) in an oribatid model mite species (without genotypic variation).

Summarizing data from **publication 1** and **2** using a Geometric Framework based analysis revealed that the reproductive fitness of *A. longisetosus* depends on dietary protein as well as fat and carbohydrates (**Figure 3a**). It appears that the intake target, which yields a maximized reproductive fitness F' is approximately 1:2.5 (protein:fat+carbohydrates; **Figure 3a**) – a ratio similar to the resources wheat grass and fungi (**Figure 1c**). This bias towards more energetic food (i.e. more carbohydrates + fat) is also true for the chemical defense C' (based on summarized data from **publication 4; Figure 3b**). The ability for chemical defense C' appears to be higher, if the mites consume fat and sugar rich diet (**Figure 3b**) – a pattern I had described before (**publication 4**) and termed the "energy-regeneration rate hypothesis". While the reproductive fitness F' was co-limited by protein and energy (**Figure 3a**), the chemical defense C' appeared to be strictly limited by energy (**Figure 3b**). This suggests that different components of *A. longisetosus*' overall fitness have different elemental (**publication 1** and **2**) as well as nutritional optima (**publication 4; Figure 3**) and trade-offs among multiple traits caused by resources can ultimately lead to trait variation (**publication 2**).

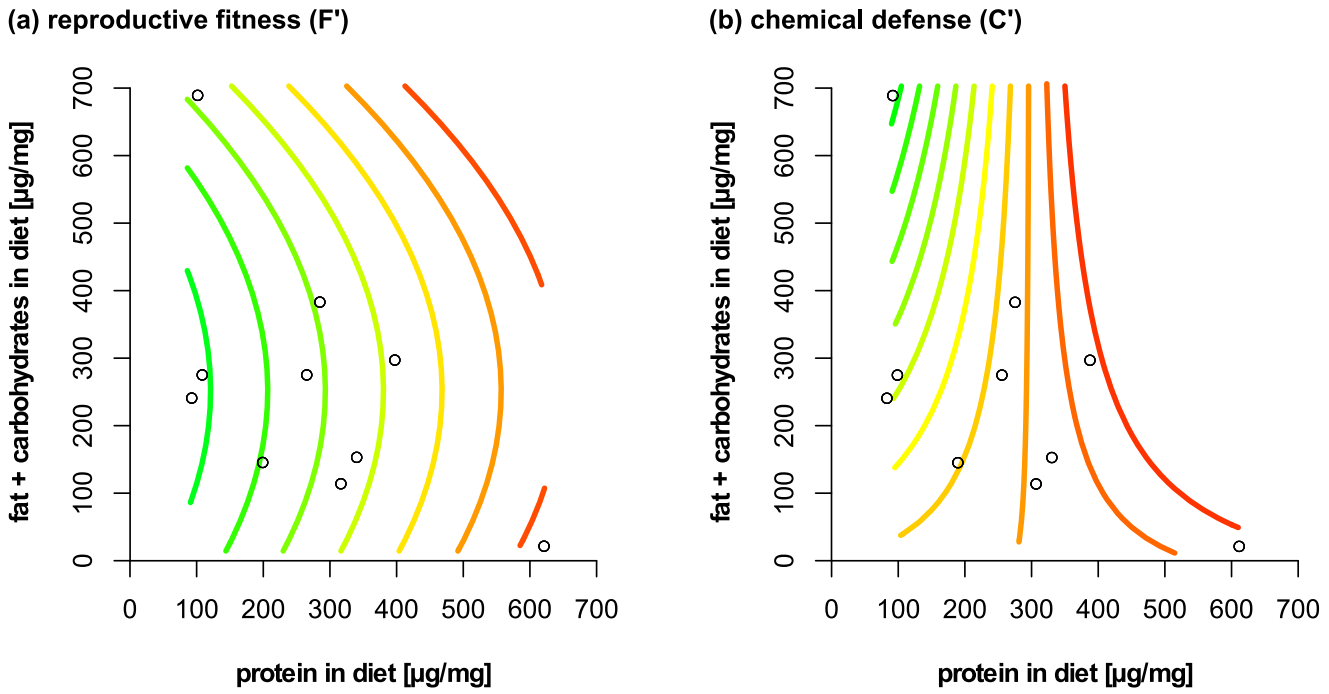


Figure 3 – The effects of macronutrient composition on fitness and chemical defense. Response surfaces of nutrient space summarizing how the protein and fat/carbohydrate content of the experimental foods affect (**a**) the reproductive fitness F′ and (**b**) the chemical defense C′ of *Archegozetes longiesotsus*. Green represents a high F′ and C′, whereas red stands for small F′ and C′. The open circles indicate the positions of the used resources in nutritional space. The lines represent model estimates of the Geometric model; concave lines point to a nutritional co-limitation, while straight lines indicate a Liebig' law for a certain nutrient or element (see Sperfeld et al., 2012). The reproductive fitness and chemical defense were calculated based on mean data extracted from **publication 2** [F = reproductive output × developmental time^{-1}] and **publication 4** [C = regeneration rate × reservoir size], respectively. Afterwards both measures were normalized to a comparable scale as F′ and C′ [$n' = (n - n_{min})/(n_{max} - n_{min})$; where n is any variable].

Applying the concept of Lailvaux and Husak (2014; **Figure 2**) to *A. longisetosus* reveals these trait-trait and trait-resource interconnections (**Figure 4**). As predicted by theory (Stearns, 1989; 2000), and similar to empirical studies on the black field cricket (Hunt et al., 2004; Lailvaux et al., 2010; Lailvaux and Kasumovic, 2011; see also summarized in Lailvaux and Husak, 2014), resource quality (herein measured as elemental ratios, **publication 1**, **2**, **7** or macronutrients; **publication 4**, **7** and **Figure 3**) affect the shape (i.e. morphometry; **publication 2**), performance (i.e. body mass; **publication 1**, **2**) and investment in reproduction (**publication 1**) on a phenotypic level (**Figure 4a**). I could show that this influences did not only manifest in altered trait means, but also in changed trait variation (**publication 2**). Moreover, I could demonstrate a trophic transfer of fatty acid from food to mites – rendering NLFAs as good biomarkers to mon-

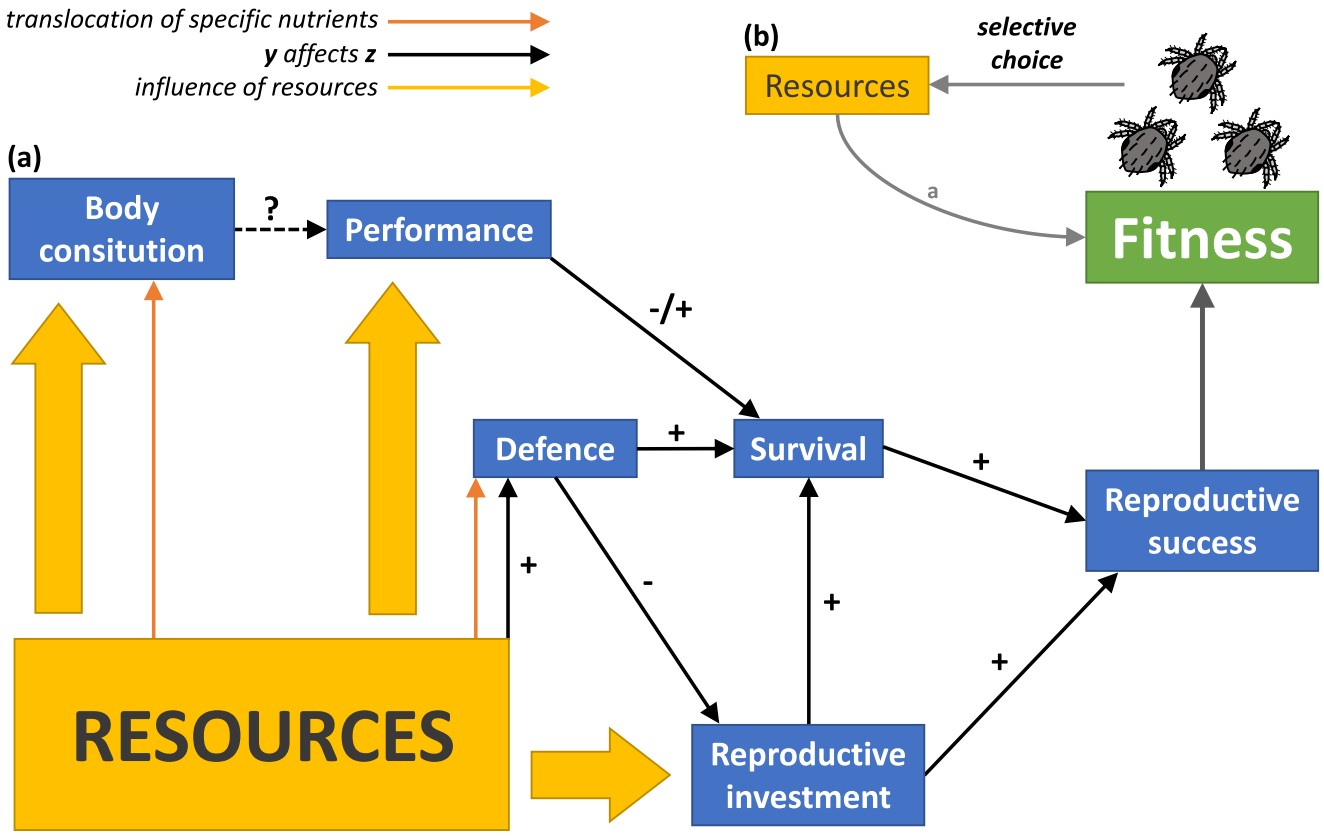


Figure 4 – The relationship of different traits and resources in the model mite *Archegozetes longisetosus*. (**a**) The revealed trait-trait and trait-resource interconnections in *A. longisetosus*. All depicted links are supported by empirical data extracted from this thesis. Body mass is used as a measure of performance (see Gillooly et al., 2001), while "body constitution" refers to the morphometry and amino-/fatty acid composition. (**b**) Fitness relevant food choice is selective and might be adaptive. The curved grey arrow summarizes all the interconnections from (**a**). Broad arrows represent a direct influence of resources on a certain trait, while thin arrows denote an effect of trait y on trait z, or the translocation of specific nutrients (but see figure insert for details). ? = unknown effect, + = positive effect, – =negative effect

itor matter flow and the connections within food webs (**publication 5**; **Figure 4a**). There was, however, no change in body stoichiometry for amino acids, suggesting a selective utilization of dietary protein (**publication 5; Figure 4a**). Regarding chemical defense, I found that sugars and fats are beneficial to maintain an effective chemical protection (**publication 4; Figure 3b**). I proved that all, but especially the highly bioactive aromatic compounds in the defensive secretions of *A. longisetosus* can be synthesized *de novo* using carbohydrate and fatty acid derived poly-β-carbonyl precursors via a polyketide reaction (**publication 6**). Given that an optimal chemical defense requires food with a high caloric value, but successful reproduction in contrast also requires a considerable amount of protein, my overall results may point to a potential negative relationship of defense and reproduction (**publication 1**, **2**, **3**, **4**, **6**; **Figure**

4a). On the other hand, chemical defense ensures longer survival of adults (**publication 4**; Heethoff et al., 2011) and juveniles (Heethoff and Raspotnig, 2012a), resulting in indirectly or directly higher reproductive success and ultimately in higher fitness (**Figure 4a**). While a performance-survival trade-off is often times theoretically predicted – offspring can either be large in mass or size (Hassall et al., 2006; Lack, 1947; Smith and Fretwell, 1974; but see Smith et al., 1989) – there was no clear evidence for this pattern in *A. longisetosus* reared on multiple resources (**Figure 4a**). Also in contrast to others (Ellers, 1995; Stearns, 1989), I did not find a trade-off between the number of offspring and the survival rate in *A. longisetosus* (**publication 1** and **2**). Consequently, both parameters are positively related to reproductive success which again ultimately leads to higher fitness. Finally, I could show that the fitness relevant choice of resources is not random (**Figure 4b**), but depends on a specifically adjusted, olfactory based sensory system (**publication 7**). Interestingly, the food selection in *A. longisetosus* was related to fatty acids (**publication 7** and **8**) – a nutrient with high caloric value, fostering the regeneration of defensive secretions (**publication 4**) – which could indicate an adaptive connection of resource selection and fitness across trophic levels (**publication 7**; **Figure 4b**). This specific olfactory-based foraging behavior appears to be relatively conserved, because I found that *A. longisetosus* did not prefer known food, but rather favored fatty acid-rich, high quality food. This indicates that food preferences are an innate and not an imprinted behavior (**publication 8**).

In summary, this thesis dealt with a lot of different topics and questions, yet all were ultimately related to a very simple, but basic problem – how does food and nutritional quality affects the life, fitness and evolutionary success of an animal under "phenotypic control". My proof-of-principle approach revealed patters and mechanisms of dietary effects on a life-history, chemo-ecological, biochemical and behavioral level and support *A. longiestosus* as a tractable model species to further study the phenotypic changes and related consequences of more specified diets, for example using the Geometric Framework of Nutrition.

6 References

Anderson, T. R., Boersma, M. and Raubenheimer, D. (2004). Stoichiometry: linking elements to biochemicals. Ecology 85, 1193-1202.

Bertrand, G. (1912). On the role of trace substances in agriculture. Eighth International Congress Applied Chem 28, 30-40.

Birch, A. J. and Donovan, F. W. (1953). Studies in relation to biosynthesis. I. Some possible routes to derivatives of Orcinol and Phloroglucinol. Australian Journal of Chemistry 6, 360-368.

Birkhofer, K., Bylund, H., Dalin, P., Ferlian, O., Gagic, V., Hambäck, P. A., Klapwijk, M., Mestre, L., Roubinet, E. and Schroeder, M. (2017). Methods to identify the prey of invertebrate predators in terrestrial field studies. Ecology and Evolution.

Blum, M. S. (1996). Semiochemical parsimony in the Arthropoda. Annual Reviews in Entomology 41, 353-74.

Bodner, M. and Raspotnig, G. (2012). Millipedes that smell like bugs: (E)-alkenals in the defensive secretion of the julid diplopod *Allajulus dicentrus*. Journal of Chemical Ecology 38, 547-56.

Boersma, M. and Elser, J. J. (2006). Too much of a good thing: on stoichiometrically balanced diets and maximal growth. Ecology 87, 1325-1330.

Bolnick, D. I., Amarasekare, P., Araújo, M. S., Bürger, R., Levine, J. M., Novak, M., Rudolf, V. H., Schreiber, S. J., Urban, M. C. and Vasseur, D. A. (2011). Why intraspecific trait variation matters in community ecology. Trends in Ecology and Evolution 26, 183-192.

Booth, R. and Anderson, J. (1979). The influence of fungal food quality on the growth and fecundity of *Folsomia candida* (Collembola: Isotomidae). Oecologia 38, 317-323.

Boutin, S. (1990). Food supplementation experiments with terrestrial vertebrates: patterns, problems, and the future. Canadian Journal of Zoology 68, 203-220.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. and West, G. B. (2004). Toward a metabolic theory of ecology. Ecology 85, 1771-1789.

Brückner, A., Raspotnig, G., Wehner, K., Meusinger, R., Norton, R. A. and Heethoff, M. (2017). Storage and release of hydrogen cyanide in a chelicerate (*Oribatula tibialis*). Proceedings of the National Academy of Sciences of the United States of America 114, 3469-3472.

Brückner, A., Wehner, K., Neis, M. and Heethoff, M. (2016). Attack and defense in a gamasid-oribatid mite predator-prey experiment - sclerotization outperforms chemical repellency. Acarologia 56, 451-461.

De Lillo, E., Nuzzaci, G. and Di Palma, A. (2004). Sensorial structures in mites and perspectives of research. Phytophaga 14, 59-81.

Ehnes, R. B., Pollierer, M. M., Erdmann, G., Klarner, B., Eitzinger, B., Digel, C., Ott, D., Maraun, M., Scheu, S. and Brose, U. (2014). Lack of energetic equivalence in forest soil invertebrates. Ecology 95, 527-537.

Ellers, J. (1995). Fat and eggs: an alternative method to measure the trade-off between survival and reproduction in insect parasitoids. Netherlands Journal of Zoology 46, 227-235.

Elser, J. J. (2003). Biological stoichiometry: a theoretical framework connecting ecosystem ecology, evolution, and biochemistry for application in astrobiology. International Journal of Astrobiology 2, 185-193.

Elser, J. J., Dobberfuhl, D. R., MacKay, N. A. and Schampel, J. H. (1996). Organism size, life history, and N: P stoichiometry. BioScience 46, 674-684.

Estrada-Venegas, E. G., Norton, R. A., Equihua-Martínez, A., Nápoles, R., Trinidad Santos, J. and González Hernandez, H. (1999). Biologia y nueva sinonima de *Archegozetes longisetosus* Aoki (Acari - Oribatida) de la Mancha, Veracruz, Mexico. Folia Entomologica Mexico 107, 41-50.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. and Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. Science 293, 2248-2251.

Haq, M. A. and Adolph, C. (1981). A comparative study of the duration of the life cycles of four species of oribatid mites (Acari: Oribatida) from the soils of Kerala. Indian Journal of Acarology 5, 56-61.

Haq, M. A. and Prabhoo, N. R. (1977). Observations on the feeding habits of oribatid mites from the soils of Kerala (Acarina: Cryptostigmata) —pahphytophages. Entomon 1, 133-137.

Hassall, M., Walters, R., Telfer, M. and Hassall, M. (2006). Why does a grasshopper have fewer, larger offspring at its range limits? Journal of Evolutionary Biology 19, 267-276.

Heethoff, M. (2012). Regeneration of complex oil-gland secretions and its importance for chemical defense in an oribatid mite. Journal of Chemical Ecology 38, 1116-23.

Heethoff, M., Bergmann, P., Laumann, M. and Norton, R. A. (2013). The 20th anniversary of a model mite: A review of current knowledge about *Archegozetes longisetosus* (Acari, Oribatida). Acarologia 53, 353-368.

Heethoff, M., Koerner, L., Norton, R. A. and Raspotnig, G. (2011). Tasty but protected-first evidence of chemical defense in oribatid mites. Journal of Chemical Ecology 37, 1037-1043.

Heethoff, M. and Rall, B. C. (2015). Reducible defence: chemical protection alters the dynamics of predator–prey interactions. Chemoecology 25, 53-61.

Heethoff, M. and Raspotnig, G. (2012a). Expanding the 'enemy-free space' for oribatid mites: evidence for chemical defense of juvenile *Archegozetes longisetosus* against the rove beetle *Stenus juno*. Experimental and Applied Acarology 56, 93-97.

Heethoff, M. and Raspotnig, G. (2012b). Triggering chemical defense in an oribatid mite using artificial stimuli. Experimental and Applied Acarology 56, 287-295.

Heidemann, K., Scheu, S., Ruess, L. and Maraun, M. (2011). Molecular detection of nematode predation and scavenging in oribatid mites: laboratory and field experiments. Soil Biology and Biochemistry 43, 229-236.

Hertweck, C. (2009). The biosynthetic logic of polyketide diversity. Angewandte Chemie International Edition 48, 4688-716.

Holliday, A. E., Walker, F. M., Brodie, E. D. and Formica, V. A. (2009). Differences in defensive volatiles of the forked fungus beetle, *Bolitotherus cornutus*, living on two species of fungus. Journal of Chemical Ecology 35, 1302-1308.

Houle, D. (1991). Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. Evolution 45, 630-648.

Hunt, J., Brooks, R., Jennions, M. D., Smith, M. J., Bentsen, C. L. and Bussiere, L. F. (2004). High-quality male field crickets invest heavily in sexual display but die young. Nature 432, 1024.

Jensen, K., Mayntz, D., Toft, S., Raubenheimer, D. and Simpson, S. J. (2011). Prey nutrient composition has different effects on *Pardosa* wolf spiders with dissimilar life histories. Oecologia 165, 577-83.

Jones, C. G., Hess, T. A., Whitman, D. W., Silk, P. J. and Blum, M. S. (1986). Idiosyncratic variation in chemical defenses among individual generalist grasshoppers. Journal of Chemical Ecology 12, 749-761.

Jones, C. G., Hess, T. A., Whitman, D. W., Silk, P. J. and Blum, M. S. (1987). Effects of diet breadth on autogenous chemical defense of a generalist grasshopper. Journal of Chemical Ecology 13, 283-297.

Jones, C. G., Whitman, D. W., Compton, S. J., Silk, P. J. and Blum, M. S. (1989). Reduction in diet breadth results in sequestration of plant chemicals and increases efficacy of chemical defense in a generalist grasshopper. Journal of Chemical Ecology 15, 1811-1822.

Jørgensen, H. B., Hedlund, K. and Axelsen, J. A. (2008). Life-history traits of soil collembolans in relation to food quality. Applied Soil Ecology 38, 146-151.

Kay, A. D., Ashton, I. W., Gorokhova, E., Kerkhoff, A. J., Liess, A. and Litchman, E. (2005). Toward a stoichiometric framework for evolutionary biology. Oikos 109, 6-17.

Krause, S., Le Roux, X., Niklaus, P. A., Van Bodegom, P. M., Lennon, J. T., Bertilsson, S., Grossart, H.-P.,

Philippot, L. and Bodelier, P. L. (2014). Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. Frontiers in Microbiology 5, 251.

Kullenberg, B., Bergstrom, G. and Stallberg-Stenhagen, S. (1970). Volatile components of the cephalic marking secretion of male bumble bees. Acta Chemica Scandinavica 24, 1481-5.

Kuwahara, Y. (2004). Chemical ecology of astigmatid mites. In Advances in Insect Chemical Ecology, eds. R. T. Cardé and J. G. Millar), pp. 76-109. Cambridge: Cambridge University Press.

Lack, D. (1947). The significance of clutch-size. Ibis 89, 302-352.

Lailvaux, S. P., Hall, M. D. and Brooks, R. C. (2010). Performance is no proxy for genetic quality: trade-offs between locomotion, attractiveness, and life history in crickets. Ecology 91, 1530-1537.

Lailvaux, S. P. and Husak, J. F. (2014). The life history of whole-organism performance. The Quarterly Review of Biology 89, 285-318.

Lailvaux, S. P. and Kasumovic, M. M. (2011). Defining individual quality over lifetimes and selective contexts. Proceedings of the Royal Society of London B: Biological Sciences 278, 321-328.

Lennox, J. G. and Wilson, B. E. (1994). Natural selection and the struggle for existence. Studies in History and Philosophy of Science Part A 25, 65-80.

Luxton, M. (1972). Studies on Oribatid Mites of a Danish Beech Wood Soil .1. Nutritional Biology. Pedobiologia 12, 434-463.

Luxton, M. (1975). Studies on the oribatid mites of a Danish beech wood soil. II. Biomass, calorimetry, and respirometry. Pedobiologia 15, 161-200.

Maraun, M., Erdmann, G., Fischer, B. M., Pollierer, M. M., Norton, R. A., Schneider, K. and Scheu, S. (2011). Stable isotopes revisited: Their use and limits for oribatid mite trophic ecology. Soil Biology and Biochemistry 43, 877-882.

Maraun, M., Schatz, H. and Scheu, S. (2007). Awesome or ordinary? Global diversity patterns of oribatid mites. Ecography 30, 209-216.

Maraun, M. and Scheu, S. (2000). The structure of oribatid mite communities (Acari, Oribatida): Patterns, mechanisms and implications for future research. Ecography 23, 374-383.

Mayntz, D., Raubenheimer, D., Salomon, M., Toft, S. and Simpson, S. J. (2005). Nutrient-specific foraging in invertebrate predators. Science 307, 111-113.

Morgan, E. D. (2010). Biosynthesis in Insects. Camebridge: RSC Publishing.

Norton, R. A. (1994). Evolutionary aspects of oribatid mite life histories and consequences for the origin of the Astigmata. In Mites: Ecological and evolutionary analyses of life-history patterns, (ed. M. A.

Houck), pp. 99-135. New York: Chapman and Hall.

Norton, R. A. (2007). Holistic acarology and ultimate causes: examples from the oribatid mites. In Acarology XI: Proceedings of the International Congress., vol. 1 eds. J. B. Morales-Malacara V. Behan-Pelletier E. Ueckermann T. M. Perez E. G. Estrada-Venegas and M. Badii), pp. 3-20. Mexico: Sociedad Latinoamericana de Acarologia.

Norton, R. A., Bonamo, P. M., Grierson, J. D. and Shear, W. A. (1988). Oribatid mite fossils from a terrestrial Devonian deposit near Gilboa, New York. Journal of Paleontology 62, 259-269.

Nunney, L. (1996). The response to selection for fast larval development in Drosophila melanogaster and its effect on adult weight: an example of a fitness trade-off. Evolution 50, 1193-1204.

Olofsson, H., Ripa, J. and Jonzén, N. (2009). Bet-hedging as an evolutionary game: the trade-off between egg size and number. Proceedings of the Royal Society of London B: Biological Sciences 276, 2963-2969.

Pankewitz, F. and Hilker, M. (2008). Polyketides in insects: ecological role of these widespread chemicals and evolutionary aspects of their biogenesis. Biological Reviews 83, 209-226.

Peschel, K., Norton, R. A., Scheu, S. and Maraun, M. (2006). Do oribatid mites live in enemy-free space? Evidence from feeding experiments with the predatory mite *Pergamasus septentrionalis*. Soil Biology and Biochemistry 38, 2985-2989.

Raspotnig, G. (2006). Chemical alarm and defence in the oribatid mite *Collohmannia gigantea* (Acari: Oribatida). Experimental and Applied Acarology 39, 177-194.

Raspotnig, G. (2010). Oil gland secretions in Oribatida (Acari). In Trends in Acarology, eds. M. W. Sabelis and J. Bruin), pp. 235-239. Dordrecht: Springer.

Raspotnig, G., Norton, R. A. and Heethoff, M. (2011). Oribatid mites and skin alkaloids in poison frogs. Biology Letters 7, 555-556.

Raubenheimer, D., Lee, K. and Simpson, S. (2005). Does Bertrand's rule apply to macronutrients? Proceedings of the Royal Society of London B: Biological Sciences 272, 2429-2434.

Raubenheimer, D., Mayntz, D., Simpson, S. J. and Tøft, S. (2007). Nutrient-specific compensation following diapause in a predator: implications for intraguild predation. Ecology 88, 2598-2608.

Raubenheimer, D. and Simpson, S. (1993). The geometry of compensatory feeding in the locust. Animal Behaviour 45, 953-964.

Raubenheimer, D. and Simpson, S. J. (1997). Integrative models of nutrient balancing: application to insects and vertebrates. Nutrition Research Reviews 10, 151-79.

Raubenheimer, D. and Simpson, S. J. (2009). Nutritional PharmEcology: doses, nutrients, toxins, and

medicines. Integrative and Comparative Biology 49, 329-337.

Riha, G. (1951). Zur Ökologie der Oribatiden in Kalksteinböden. Zoologische Jahrbücher 80, 407-450.

Rosumek, F. B., Brückner, A., Blüthgen, N., Menzel, F. and Heethoff, M. (2017). Patterns and dynamics of neutral lipid fatty acids in ants–implications for ecological studies. Frontiers in Zoology 14, 36.

Ruess, L. and Chamberlain, P. M. (2010). The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biology and Biochemistry 42, 1898-1910.

Ruess, L., Häggblom, M. M., Zapata, E. J. G. a. and Dighton, J. (2002). Fatty acids of fungi and nematodes—possible biomarkers in the soil food chain? Soil Biology and Biochemistry 34, 745-756.

Saporito, R. A., Donnelly, M. A., Norton, R. A., Garraffo, H. M., Spande, T. F. and Daly, J. W. (2007). Oribatid mites as a major dietary source for alkaloids in poison frogs. Proceedings of the National Academy of Sciences of the USA 104, 8885-8890.

Saporito, R. A., Donnelly, M. A., Spande, T. F. and Garraffo, H. M. (2011). A review of chemical ecology in poison frogs. Chemoecology 22:159-168.

Schaefer, I., Norton, R. A., Scheu, S. and Maraun, M. (2010). Arthropod colonization of land–Linking molecules and fossils in oribatid mites (Acari, Oribatida). Molecular Phylogenetics and Evolution 57, 113-121.

Schatz, H., Behan-Pelletier, V. M., O'Connor, B. M. and Norton, R. A. (2011). Suborder Oribatida van der Hammen, 1968. In Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness", vol. 3148 (ed. Z.-Q. Zhang), pp. 141-148: Zootaxa.

Schlichting, C. D. and Pigliucci, M. (1998). Phenotypic evolution: a reaction norm perspective: Sinauer Associates Incorporated.

Schneider, K., Renker, C., Scheu, S. and Maraun, M. (2004). Feeding biology of oribatid mites: a minireview. Phytophaga 14, 247-256.

Schuster, R. (1956). Der Anteil der Oribatiden an den Zersetzungsvorgängen im Boden. Zeitschrift für Morphologie und Ökologie der Tiere 45, 1-33.

Schuster, R. (1979). Soil mites in the marine environment. Recent Advances in Acarology 1, 593-602.

Seniczak, A. (1998). Preliminary studies on the influence of food on the development and morphology of Archegozetes longisetosus Aoki (Acari, Oribatida) in laboratory conditions. Zeszyty Naukowe Akademii Techniczno-Rolniczej w Bydgoszczy. Ochrona Srodowiska (Poland) 2, 175-180.

Seniczak, A. (2006). The effect of density on life-history parameters and morphology of Archegozetes longisetosus Aoki, 1965 (Acari: Oribatida) in laboratory conditions. Biological Letters 43, 209-213.

Seniczak, A., Ligocka, A., Seniczak, S. and Paluszak, Z. (2016). Effects of green algae and napa cabbage on life-history parameters and gut microflora of *Archegozetes longisetosus* (Acari: Oribatida) under laboratory conditions. Biological Letters 53, 67-78.

Shear, W. A., Bonamo, P. M., Grierson, J. D., Rolfe, W. I., Smith, E. L. and Norton, R. A. (1984). Early land animals in North America: evidence from Devonian age arthropods from Gilboa, New York. Science 224, 492-494.

Shimano, S., Sakata, T., Mizutani, Y., Kuwahara, Y. and Aoki, J.-i. (2002). Geranial: the alarm pheromone in the nymphal stage of the oribatid mite, Nothrus palustris. Journal of Chemical Ecology 28, 1831-1837.

Simpson, S. and Raubenheimer, D. (1993). A multi-level analysis of feeding behaviour: the geometry of nutritional decisions. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 342, 381-402.

Simpson, S. J. and Raubenheimer, D. (2012). The nature of nutrition: a unifying framework from animal adaptation to human obesity: Princeton University Press.

Simpson, S. J., Sibly, R. M., Lee, K. P., Behmer, S. T. and Raubenheimer, D. (2004). Optimal foraging when regulating intake of multiple nutrients. Animal Behaviour 68, 1299-1311.

Smith, C. C. and Fretwell, S. D. (1974). The optimal balance between size and number of offspring. The American Naturalist 108, 499-506.

Smith, H. G., Kallander, H. and Nilsson, J.-A. (1989). The trade-off between offspring number and quality in the great tit Parus major. The Journal of Animal Ecology, 383-401.

Smrž, J. (1992). Some adaptive features in the microanatomy of moss-dwelling oribatid mites (Acari: Oribatida) with respect to their ontogenetical development. Pedobiologia 36, 306-320.

Smrž, J. (2010). Nutritional biology of oribatid mites from different microhabitats in the forest. In Trends in Acarology, pp. 213-216: Springer.

Smrž, J. and Norton, R. A. (2004). Food selection and internal processing in *Archegozetes longisetosus* (Acari : Oribatida). Pedobiologia 48, 111-120.

Sørensen, A., Mayntz, D., Raubenheimer, D. and Simpson, S. J. (2008). Protein-leverage in mice: the geometry of macronutrient balancing and consequences for fat deposition. Obesity 16, 566-571.

Sperfeld, E., Halvorson, H. M., Malishev, M., Clissold, F. J. and Wagner, N. D. (2016). Woodstoich III: Integrating tools of nutritional geometry and ecological stoichiometry to advance nutrient budgeting and the prediction of consumer-driven nutrient recycling. Oikos 125, 1539-1553.

Sperfeld, E., Martin-Creuzburg, D. and Wacker, A. (2012). Multiple resource limitation theory applied to

herbivorous consumers: Liebig's minimum rule vs. interactive co-limitation. Ecology Letters 15, 142-150.

Stearns, S. C. (1989). Trade-offs in life-history evolution. Functional Ecology 3, 259-268.

Stearns, S. C. (2000). Life history evolution: successes, limitations, and prospects. Naturwissenschaften 87, 476-486.

Sterner, R. W. and Elser, J. J. (2002). Ecological stoichiometry: the biology of elements from molecules to the biosphere: Princeton University Press.

Thomas, J. (1979). An energy budget for a woodland population of oribatid mites. Pedobiologia 19, 346-378.

Unruh, L. M., Xu, R. D. and Kramer, K. J. (1998). Benzoquinone levels as a function of age and gender of the red flour beetle, *Tribolium castaneum*. Insect Biochemistry and Molecular Biology 28, 969-977.

Van Amelsvoort, P. and Usher, M. (1989). Egg production related to food quality in *Folsomia candida* (Collembola: Isotomidae): effects on life history strategies. Pedobiologia 33, 61-66.

Van Noordwijk, A. J. and de Jong, G. (1986). Acquisition and allocation of resources: their influence on variation in life history tactics. The American Naturalist 128, 137-142.

Vanni, M. J. and Lampert, W. (1992). Food quality effects on life history traits and fitness in the generalist herbivore *Daphnia*. Oecologia 92, 48-57.

Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. and Garnier, E. (2007). Let the concept of trait be functional! Oikos 116, 882-892.

Wallwork, J. (1979). Energetics of soil mites: the experimental approach. In Proceedings of the 4th International Congress of Acarology, Saalfelden (Austria)/edited by Edward Piffl: Budapest, Akademiai Kiado, 1979.

Walsh, C. and Tang, Y. (2017). Natural product biosynthesis : chemical logic and enzymatic machinery. London: Royal Society of Chemistry.

Wehner, K., Norton, R. A., Blüthgen, N. and Heethoff, M. (2016). Specialization of oribatid mites to forest microhabitats—the enigmatic role of litter. Ecosphere 7, ecs2.1336.

Whitman, D. W. and Agrawal, A. A. (2009). What is phenotypic plasticity and why is it important. Phenotypic plasticity of insects: Mechanisms and consequences, 1-63.

Whitman, D. W., Jones, C. G. and Blum, M. S. (1992). Defensive secretion production in lubber grasshoppers (Orthoptera, Romaleidae) - influence of age, sex, diet, and discharge frequency. Annals of the Entomological Society of America 85, 96-102.

Woltereck, R. (1909). Weitere experimenelle Untersuchungen über Artveränderung, speziell uber des We-

sen quantitativer Artunterschiede bei Daphniden. Verhandlungen der Deutsche Zoologischen Gesellschaft 19, 110-172.

Zachvatkin, A. A. (1941). Fauna of the U.S.S.R., Vol. VI, No.1 Tyroglyphoidae (Acari). Zoological Institute of the Acaemy of Science of the U.S.S.R., Moscow.

Zera, A. J. and Harshman, L. G. (2001). The physiology of life history trade-offs in animals. Annual Review of Ecology and Systematics 32, 95-126.

7 Declaration - Ehrenwörtliche Erklärung

Ich erkläre hiermit ehrenwörtlich, dass ich die vorliegende Arbeit entsprechend den Regeln guter wissenschaftlicher Praxis selbstständig und ohne unzulässige Hilfe Dritter angefertigt habe.

Sämtliche aus fremden Quellen direkt oder indirekt übernommenen Gedanken sowie sämtliche von Anderen direkt oder indirekt übernommenen Daten, Techniken und Materialien sind als solche kenntlich gemacht. Die Arbeit wurde bisher bei keiner anderen Hochschule zu Prüfungszwecken eingereicht.

Darmstadt, den 21. März 2018

_____

(Adrian Brückner)

8 Acknowledgements

I am in debt to lots of individuals who made this work possible, yet I would like to give special credit to:

Michael Heethoff. Thanks Micha for having me as your PhD student. It was certainly not always easy for you given that we exchanged about 1500 emails, and that I spent at least 1500 hours in your office talking, asking questions, learning and becoming a better scientist. Thank you for all your patience, ideas, positive criticisms, the conferences, the freedom to do a lot of other things – I am so grateful and indebt to you.

Andreas Jürgens. Thanks Andreas for being part of my committee as the co-examiner of this thesis. Discussing chemical ecology, but also science-philosophy problems with you was always a pleasure.

Nico Blüthgen. Thanks Nico for having me as an "Examenskandidat" in the first place. You opened "the door to science" for me and gave me the chance to be the scientist I never thought I would be. I am grateful for your new ideas, your (statistical) support and encouragement over the past five years. Finally thanks for being part of my committee.

Günther Raspotnig. Thanks Günther, you introduced me to the chemical ecology of oribatid mites in the first place and taught me a lot about these incredible animals I grew so fond of. I have to thank you for the time in Graz which was seminal for me and my future work. It is great to have you as a part of my committee.

Reinhard Meusinger. Thanks Reinhard for participating in some of my projects, for sharing your wisdom about NMR and for being the "foreign"-examiner in my committee.

Studienstiftung des deutschen Volkes. Thanks goes also to the SdV for financing me for 8 years during my undergraduate and PhD studies. This support enabled me to start a PhD at all, to study aboard and to travel to many interesting conferences all around the world.

Roy A. Norton. Thanks Roy for your guidance and patience, for proof reading so many manuscripts, for answering silly question via email, for introducing me to even more fascinating oribatid mites at OSU and for giving me the feeling that I do some really interesting stuff. And finally of course thanks for collecting Archegozetes longisetosus in Puerto Rico 25 years ago.

Christoph von Beeren. Thanks Chris for being my guide to the ant world, for bringing me to Costa Rica, for supporting me and of course for our work together. I could write so much more, but I will just say: "spitzenmäßig".

Kevin Frank. Thanks Kevin for being my office mate for five years; a time you survived surprisingly well. I could not image a better guy to procrastinate (i.e., drinking coffee, talking about series/computer game or complaining about ourselves and others), but also to do weird science. With dung. And Beetles.

Katja Wehner. Thanks Katja for all your help with some of the most essential experiments of this work – without your help this thesis would not have been possible. It was fun sitting in the small basement room with you for nearly two month counting mites.

Sebastian Schmelzle. Thanks Sib for not protesting when Kevin and I "conquered" the office. Thanks for all the IT-support, the "pixel"-science, your constructive criticism, for scanning my documents, for tolerating my talkative presence and of course for fostering my coffee consume.

Félix B. Rosumek. Thanks Félix for being such an awesome colleague; you taught me a lot about Brazil, the pleasure of Friday-after-work bear and ants. It was a pleasure to be a little part of your project as well, maybe we can keep it this way – there are much more fat ants to explore.

All others who work or worked in the „Ecological Networks"-group over the past years (in alphabetic order): **Alba Costa**, **Andrew Bruce**, **Anne Happe**, **Arturo Lonighi**, **Christopher Kaiser-Bunbury**, **Fernanda Costa**, **Inácio Teles**, **Jule Mangels**, **Karsten Mody** and **Melanie**

Chisté – thanks creating such a homely place to work.

Karin Römer. Thanks Karin for taking care of all the "Auszahlung unbar", "Reisekostenabrechnungen" and so much more administrative stuff.

Andrea Hilpert. Thanks Andrea for all the encouragement, coffee and the bike tours back to Griesheim. You not only analyzed the amino acids for one of my papers, you also forced me to keep my stuff tied up – thank you!

I am also in debt to all the Bachelor/experimental ecology-module students (**Max Maschler**, **Max Neis**, **Romina Schuster**, **Timo Smit**, **Christoph Merkel** and **Jascha Höpfner**) who were somehow involved in one of my projects. I especially want to thank **Romina Schuster** who digitalized about 20,000 rows of data and counted/weighted several thousand mites. Hopefully, I was not too sarcastic and you learned some interesting stuff about mites and chemical ecology (at least I tried).

Many thanks to all my collaborators, who were somehow involved in my research over the past years (in alphabetic order): **Florian Menzel**, **Hans Klompen**, **Irmgard Schäffler**, **Mario Schubert**, **Martin Kaltenpoth**, **Melanie M. Pollierer** and **Stefan Dötterl** – thanks to you all for your time and knowledge.

One huge "Thank you" to my flat mates **Tim**, **Danny** and **Marc** (as well as our alumnus **Patrick**) for tolerating my weirdness, my talkative nature and for being my friends. A special hug and gratitude for **Tim**, who not only helped me to layout and typeset this thesis, but is also one of the finest person I am privileged to call a friend. Good luck guys, the next crazy biologist is just about to arrive – **Phil**, have fun, but be gentle ;)

My family. Because of you, I am who I am - without you, I would be nothing.

Finally, I have to thank ***Archegozetes longisetosus*** for being such a magnificent and fascinating animal - without her help this thesis would obviously not exist.

9 Curriculum vitae

Personal details

Name	Adrian Brückner
Date of birth	30th December 1989
Place of birth	Alzenau, Germany
Nationality	German

Education

05/2015 – 05/2018: PhD student at TU Darmstadt, Germany. Thesis: "Resources and oribatid mites – effects on life history, chemical ecology, biochemistry and food selection"; supervisor: PD Dr. Michael Heethoff

04/2015: State Examination in Biology and Chemistry (1. Staatsexamen) at the TU Darmstadt, Germany

07/2014 – 11/2014: Research student at Karl-Franzens-University in Graz, Austria; host: PD Dr. Günther Raspotnig

08/2013 – 06/2014: State examination-thesis at the TU Darmstadt, Germany: "Water loss of flower visitors"; supervisor: Prof. Dr. Nico Blüthgen

10/2010 - 05/2015: Student of Biology and Chemistry (for secondary school teachers) at TU Darmstadt, Germany

Publications

Publications which are part of this thesis are written in **bold**, others are in *italics*.

(25) Brückner A., Kaltenpoth M., Heethoff M. (2018) *De novo* biosynthesis of simple aromatic compounds by an arthropod (*Archegozetes longisetosus*). PNAS (submitted)

(24) Brückner A., Schuster R., Wehner K., Heethoff M. (2018) Nutritional quality modulates intraspecific trait variability. Front Zool (submitted)

(23) Brückner A., Heethoff M., Norton R.A., Wehner K. (2018) Body size structure of oribatid mite

communities in different microhabitats. Int J Acarol (submitted)

(22) Heethoff M., Brückner A.*, Schmelzle S.*, Schubert M.*, Bräuer M., Meusinger R., Dötterl S., Norton R.A., Raspotnig G. (2018) Life as a fortress – Structure, function, and adaptive values of morphological and chemical defense in the oribatid mite* Euphthiracarus reticulatus *(Actinotrichida). BMC Zoology (revised) *=joined first authors*

(21) Frank K., Brückner A., Blüthgen N., Schmitt T. (2018) In search for cues: dung beetle attraction and the significance of volatile composition in dung. Chemoecology (revised)

(20) Brückner A., Hoenle P.O., von Beeren C. (2018) Mandibular gland chemistry of Eciton *army ants: a chemo-evolutionary perspective. PeerJ (submitted)*

(19) Wehner K., Heethoff M., Brückner A. (2018) Seasonal fluctuation of oribatid mite communities in forest microhabitats. PeerJ (in press)

(18) Brückner A., Schuster R., Smit T., Heethoff M. (2018) Imprinted or innate food preferences in the model mite *Archegozetes longisetosus* (Actinotrichida, Oribatida, Trhypochthoniidae). Soil Org 90: 23-26

(17) Wehner K., Heethoff M., Brückner A. (2018) Sex ratios of oribatid mites differ among microhabitats. Soil Org 90: 13-22

(16) Brückner A., Schuster R., Wehner K., Heethoff M. (2018) Effects of nutritional quality on the reproductive biology of *Archegozetes longisetosus* (Actinotrichida, Oribatida, Trhypochthoniidae). Soil Org 90: 1-12

(15) von Beeren C., Brückner A., Maruyama M., Burke G., Wieschollek J., Kronauer J.C. (2018). Chemical and behavioral integration of army ant-associated rove beetles - a comparison between specialists and generalists. Front Zool 15: 8

(14) Brückner A., Heethoff M. (2018). Nutritional effects on chemical defense alter predator-prey dynamics. Chemoecology 28: 1-9

(13) Brückner, A., Schuster, R., Smit, T., Pollierer, M.M., Schäffler, I., Heethoff, M. (2018)

Track the snack - olfactory cues shape foraging behaviour of decomposing soil mites (Oribatida). Pedobiologia 66: 74-80

(12) Brückner A., Klompen H., Bruce A.I., Hashim R., von Beeren C. (2017) Infection of army ant pupae by two new parasitoid mites (Mesostigmata: Uropodina). PeerJ 5:e3870

(11) Kühsel S., Brückner A., Schmelzle S., Heethoff M., Blüthgen N. (2017) Surface area – volume ratios in insects. Insect Sci 24: 829-841

(10) Frank K., Brückner A.*, Hilpert A., Heethoff M., Blüthgen N. (2017) Nutrient quality of vertebrate dung as a diet for dung beetles. Sci Rep 7:12141 *=joined first authors*

(9) Brückner A., Hilpert A., Heethoff M. (2017) Biomarker function and nutritional stoichiometry of neutral lipid fatty acids and amino acids in oribatid mites. Soil Biol Biochem 115:35-43.

(8) Rosumek F.B., Brückner A.*, Blüthgen N., Menzel F., Heethoff M. (2017) Patterns and dynamics of neutral lipid fatty acids in ants - implications for ecological studies. Front Zool 14:36 *=joined first authors*

(7) Brückner A., Heethoff M. (2017) The ontogeny of oil gland chemistry in the oribatid mite *Archegozetes longisetosus* Aoki (Oribatida, Trhypochthoniidae). Int J Acarol 43(5): 337-342

(6) Brückner, A., Heethoff, M., Blüthgen, N. (2017) The relationship between epicuticular long-chained hydrocarbons and surface area - volume ratios in insects (Diptera, Hymenoptera, Lepidoptera). Plos One 12: e0175001

(5) Brückner, A., Raspotnig, G.*, Wehner, K., Meusinger, R., Norton, R.A., Heethoff, M. (2017) Storage and release of hydrogen cyanide in a chelicerate (Oribatula tibialis). PNAS 114(13): 3469-3472 *=joined first authors*

(4) Brückner A., Heethoff M. (2017) A chemo-ecologists' practical guide to compositional data analysis. Chemoecology 27: 33-46

(3) Brückner A., Wehner K., Neis M., Heethoff M. (2016) Attack and defense in a gamasid-oribatid mite predator-prey experiment – sclerotization outperforms chemical repellency. Acarologia 56: 451-461

(2) Brückner A., Heethoff M. (2016) Scent of a mite – Origin and chemical characterization of the lemonlike flavor of mite-ripened cheeses. Exp Appl Acarol 69: 249-261

(1) Brückner A., Stabentheiner E., Leis H.-J., Raspotnig G. (2015) Chemical basis of unwettability in Liacaridae (Acari, Oribatida): specific variations of a cuticular acid/ester-based system. Exp Appl Acarol 66(3): 313-335

Grants/Scholarship/Prizes

08/2015 – 05/2018: PhD Scholarship for graduate students; German National Academic Foundation (Studienstiftung des deutschen Volkes)

09/2017: Poster award at the 110th meeting of the German Zoological Society in Bielefeld; Deutsche Zoologische Gesellschaft

08/2017: Student travel prize to ISCE 207, Kyoto, Japan; International Society of Chemical Ecology

06/2016: Acarology Summer Program, George W. Wharton fellowship 2016; Ohio State University

02/2016: Traveling grant; German National Academic Foundation (Studienstiftung des deutschen Volkes)

11/2010 – 05/2015: Scholarship for undergraduate students; German National Academic Foundation (Studienstiftung des deutschen Volkes)

10 Chapters

10.1 Life history

10.1.1 Publication 1: **Effects of nutritional quality on the reproductive biology of *Archegozetes longisetosus* (Actinotrichida, Oribatida, Trhypochthoniidae)**

Adrian Brückner, Romina Schuster, Katja Wehner and Michael Heethoff

Published in Soil Organisms 90: 1-12

Submitted: 27.11.2017, Accepted: 06.03.2018, Published: 01.04.2018

Authors contributions:

AB and MH designed the research; AB, RS and KW performed the experiment, AB performed chemical analyses; AB analyzed the data; AB and MH wrote the paper. All authors discussed and approved the final manuscript.

Reprinted by permission from Senckenberg Museum of Natural History Görlitz. Soil Organisms. Effects of nutritional quality on the reproductive biology of Archegozetes longisetosus (Actinotrichida, Oribatida, Trhypochthoniidae). Brückner Adrian, Schuster Romina, Wehner Katja, Heethoff Michael. © Senckenberg Museum of Natural History Görlitz 2018

SOIL ORGANISMS 90 (1) · April 2018 pp. 1–12

Effects of nutritional quality on the reproductive biology of *Archegozetes longisetosus* (Actinotrichida, Oribatida, Trhypochthoniidae)

Adrian Brückner*, Romina Schuster, Katja Wehner and Michael Heethoff*

Ecological Networks, Technische Univeristät Darmstadt, Schnittspahnstraße 3, 64287 Darmstadt, Germany

* Corresponding authors, e-mail: adrian.brueckner@gmail.com, heethoff@bio.tu-darmstadt.de

Received 27 November 2017 | Accepted 6 March 2018
Published online at www.soil-organisms.de 1 April 2018 | Printed version 15 April 2018

Abstract

The parthenogenetic trhypochthoniid oribatid mite *Archegozetes longisetosus* serves as a model organism. Numerous studies have investigated different aspects of its life history and nutritional biology, yet several results remain contradictive. To clarify effects of nutrition on life history parameters, we set up a large scale experiment with ten food resources of different origins and nutritional composition (animal, bacterial, fungal and herbal). Generally, food influenced all life history parameters. The number of offspring ranged from 0 to 106 individuals per female, while the developmental time and body mass varied in a range of 32 up to 88 days and 3 to 43 µg dry weight, respectively. The number of offspring per female was correlated to the C/N-ratio and thus the availability of nitrogen in the food, while the body mass was correlated to the C/P-ratio and C/Ca-ratio (for early juvenile instars). The developmental time did not respond to any measured nutritional parameter.

Keywords Nutrient ecology | life history | macroelements | mites | development

1. Introduction

Archegozetes longisetosus Aoki (1965) is among the most studied soil microarthropods, and certainly the best investigated oribatid mite species (Heethoff et al. 2013). It is found on continents and islands throughout the tropical regions of the world (Subias 2004) and seems to be a panphytophagous mite, mostly feeding on fungi, algae and decomposed litter (e.g., Beck 1967, Haq 1978, 1982). *Archegozetes longisetosus* predominantly occurs in anthropogenically disturbed habitats, e.g., organic trash, debris, compost or frequently timbered forest patches (see Heethoff et al. 2013). Such disturbed habitats differ from natural forests in their availability of biomass and macronutrient composition, e.g. more bacteria/animal remains and higher amounts of nitrogen (Borken et al. 2002, Bastida et al. 2008). This nutritional heterogeneity and potentially fast turnover of resources in its' preferred habitat may explain why *A. longisetosus* appears to be an opportunistic feeder in laboratory feeding experiments (Heidemann et al. 2011, Heethoff & Scheu 2016, Brückner et al. 2018a).

Published life history studies (e.g., Haq 1978, Haq & Adolph 1981, Honciuc 1996, Seniczak 1998, 2006, Seniczak et al. 2016) on *A. longisetosus*, which also discussed the direct or indirect effects of food, are conflicting. While Estrada-Venegas et al. (1999) claimed that food had no influence on the development of *A. longisetosus* individuals from a Mexican population, specimens of the well-defined laboratory strain *A. longisetosus* ran (Heethoff et al. 2007), showed considerable response of life history parameters, food processing and body size to different food (Seniczak 1998, Smrz & Norton 2004, Seniczak et al. 2016). Comparing algae, lichens and tree bark, Seniczak (1998) found an influence of food on the number of offspring (48–102

© Senckenberg Museum of Natural History Görlitz · 2018
ISSN 1864-6417 (print) · **ISSN** 2509-9523 (digital)

open access

individuals per female), developmental time (32–45 days), mortality (4–21 %), certain morphometrical features (e.g. body length: 884–1119 µg) and also sclerotization. According to this study (Seniczak 1998) higher amounts of total protein may cause a higher reproductive output, shorter developmental times and lower mortality. This, however, would be in contrast to the results of Seniczak et al. (2016), where napa cabbage (26.3 % total protein) always performed worse when compared to algae (15.5 % total protein). Hence, it is hitherto not possible to decipher the effects of nutritional composition of food on *A. longisetosus*.

To elucidate the influences of nutritional quality on the reproductive biology of *A. longisetosus* we setup a no-choice feeding experiment using ten different food resources from different origins (animal, bacterial, fungal, herbal; Tab. 1) and recorded life history parameters and body masses over a period of three months. We investigated i) the differences in life history parameters caused by different food along a complete ontogenetic sequence (from egg to adult), and ii) how macroelement composition (C, N, P, Ca) can help to understand these differences.

2. Materials and methods

2.1. Experimental setup

Archegozetes longesitosus ran (Heethoff et al. 2007) were reared at approx. 28°C and 80–85 % relative humidity in constant darkness on one out of ten resources for several generations (approx. 18 month). Detailed information about the ten resources of animal, bacteria, fungal and herbal origin can be found in Table 1. Fresh food and water was provided *ad libitum* three times a week. For each resource, specimens were cultured in three separate plastic boxes (100 × 100 × 50 mm) grounded with 2 cm mixture of plaster of Paris/activated charcoal mixture (9:1).

At the start of the experiment we randomly selected 25 specimens per resource from the original cultures (25 replicates × 10 resources = 250 experimental boxes in total), and individually placed them into small culture boxes (45 × 40 × 35 mm; grounded with the plaster of Paris mixture). The 250 mites could lay eggs for ten days (the adult individuals were removed then) and the same food and water was provided *ad libitum* three times a week. Every box was checked on a daily base and we counted the number all eggs, larvae, protonymphs, deutonymphs, tritonymphs and adults for a period of up to 12 weeks (new eclosed adults were removed to avoid new egg deposition). For each replicate we removed one individual per juvenile instar. These specimens and the initially used mothers were subsequently dried at 60°C until weight constancy (approx. 3 days) to determine their body mass (dry weight) with a microbalance (Mettler Toledo, XS3DU, Columbus, USA; with 0.1 µg). The body masses of dried eggs and larvae were always < 1 µg, thus we could not reliably determine their individual weight.

2.2. Analyses of carbon, nitrogen, phosphorus and calcium

For carbon (C) and nitrogen (N) analyses dried resource powders were weighed into tin capsules

Table 1. Summarizing table of the ten resources offered to *Archegozetes longisetosus* including food classificant, supplier and macronutrient composition. Higher ratios indicate a larger proportion of carbon (C = carbon) compared to the other elements (N = nitrogen, P = phosphorus, Ca = calcium).

classification		supplier	elemental analyses		
			C/N	C/P	C/Ca
animal	blood	Common Baits, Rosenfeld, Germany	3.3	541.6	866.6
	bone	Canina Pharma, Hamm, Germany	4.2	4.4	2.0
bacterial	spirulina	Interaquaristik, Biedenkopf, Germany	4.3	38.7	690.1
fungal	fungi	Arche Naturprodukte, Hilden, Germany	11.3	53.6	836.2
	yeast	Rapunzel Naturkost, Legau, Germany	7.5	51.1	417.9
herbal	chlorella	Naturya, Bath, UK	5.4	38.6	192.8
	hemp	Naturya, Bath, UK	5.6	26.2	264.1
	lupine	Govinda Natur, Neuhofen, Germany	7.0	79.1	305.8
	pollen	Ascopharm, Wernigerode, Germany	13.6	119.5	544.4
	wheat	Naturya, Bath, UK	12.4	183.9	176.3

(5±1 mg) and subsequently measured by an elemental analyzer (EA 1108 Elemental Analyzer, Carlo Erba, Milan, Italy). Acetanilide (Merck, Darmstadt, Germany) was used as an external standard. For phosphorus (P) and calcium (Ca) analyses 10 ± 1 g of the dried resource powders were digested using microwave-assisted pressure decomposition and subsequently measured via inductively coupled plasma atomic emission spectroscopy (ICP-AES). P and Ca analyses were performed by LUFA Nord-West (Oldenburg, Germany) according to DIN standard EN 15621:2012. C, N, P and Ca amounts were calculated based on external standards and the initial dry weight and expressed as C/N, C/P and C/Ca ratios.

2.3. Statistical analyses

The developmental time [days] for each mother's offspring was calculated as weighted arithmetic mean (developmental time= $\sum [d_i * p_i]$; where d_i is the day and p_i is the proportion of new adult specimens on d_i). The counted numbers [N] of eggs, larvae, proto-, deuto-, tritonymphs and adults, as well as the eclosion/hatching time [day] and the body mass [µg] across all resources were analyzed with Kruskal-Wallis tests (Kruskal & Wallis 1952). As post-hoc tests we used Mann-Whitney-U multiple pairwise comparisons (Mann & Whitney 1947). The differences in the number of eggs, larvae, proto-, deuto-, tritonymphs and adults within one resource were analyzed with Friedman tests (Friedman 1937) and pairwise Wilcoxon rank-sum tests (Wilcoxon 1945) to access differences between an instar pair. We used Spearman's rank coefficient (Spearman 1904) to explore whether the nutritional quality (expressed as C/N, C/P and C/Ca ratios) of the resources was correlated with the means of total number [N], eclosion time [day] or dry weight [µg]. Since only one individual reached the adult stage in the blood meal treatment, we had to exclude it from some analyses (but see results). Type I error accumulation for all analyses was corrected with the false discovery rate (Benjamini & Hochberg 1995). Additionally, we performed ordinary least squares regressions to analyse the i) increase in dry body mass [µg] from protonymph to adult and ii) the effects of mite density on body mass (see Seniczak 2006). Finally, we analysed if there is a trade-off between the total number of offspring [N] and the total development time [d] of a mothers offspring across the resources. To standardize both variables to a comparable scale we normalized both as for total number and for total development time and plotted means ± standard errors as xy-scatter. All statistics were performed with PAST 3.16 (Hammer et al. 2001) and R 3.3.2 (R Core Team 2016).

3. Results

3.1. Macroelement composition

The food offered to *Archegozetes longisetosus* differed in its macroelemental composition (Tab. 1). For example, while blood meal was extremely rich in organic N, it lacked P and Ca. Other foods like fungi, wheat and pollen had much less N, but comparatively more C, yet their proportion of P/Ca was very variable (Tab. 1).

3.2. Life history

The ten resources resulted in strong differences in the number of individuals (= offspring) across all instars of *A. longisetous* (Fig. 1A; Egg: $N = 219$, $df = 9$, $\chi^2 = 106.40$, $P < 0.0001$; Larva: $N = 217$, $df = 9$, $\chi^2 = 106.70$, $P < 0.0001$; Protonymph: $N = 196$, $df = 9$, $\chi^2 = 124.80$, $P < 0.0001$; Deutonymph: $N = 192$, $df = 9$, $\chi^2 = 129.50$, $P < 0.0001$; Tritonymph: $N = 183$, $df = 9$, $\chi^2 = 134.50$, $P < 0.0001$; Adult: $N = 173$, $df = 9$, $\chi^2 = 134.7$, $P < 0.0001$). Within each instar, blood meal (0.7 indviduals/female) and spirulina (5.6 indviduals/female) fed mites had the lowest number of offspring, while especially wheat grass (55.4 indviduals/female) animals had the highest number of offspring (see post-hoc comparisons Fig. 1A and Tab. 2 for detailed numbers). Similar to the offspring number, also eclosion/hatching times of the different instars across all food resources were significantly different (Fig. 1B; Larva: $N = 200$, $df = 8$, $\chi^2 = 41.48$, $P < 0.0001$; Protonymph: $N = 186$, $df = 8$, $\chi^2 = 38.42$, $P < 0.0001$; Deutonymph: $N = 185$, $df = 8$, $\chi^2 = 39.04$, $P < 0.0001$; Tritonymph: $N = 181$, $df = 8$, $\chi^2 = 49.29$, $P < 0.0001$; Adult: $N = 171$, $df = 8$, $\chi^2 = 41.13$, $P < 0.0001$). Mites fed with blood meal were excluded from these analyses because only one individual finally hatched to an adult and thus no statistical evaluation was possible (see also Tab. 2 for detailed numbers). Generally, the patterns of eclosion/hatching times were quite stable across all instars, just the initial period before eggs hatched was different (Fig. 1B, Tab. 2). Afterwards, the time pattern became quite similar (see post-hoc tests Fig. 1B), with hemp (18–49 days) and yeast (19–48 days) leading to the earliest hatching and bone (27–68 days), fungi (24–71 days) and spirulina (38–75 days) to the latest hatching times. As for the number of individuals, also the eclosion/hatching time of the other resources gradually differed from each other (Fig. 1 post-hoc results, Tab. 2). Also the number of eggs, larvae, proto-, deuto-, tritonymphs and adults within one resource differed, indicating some degree of mortality of the juvenile instars (Fig. 2; see also Friedman tests and Wilcoxon post-hoc results

in Tab. 2). For some food resources the mortality was relatively high (e.g., yeast ~50%, chlorella ~ 90% and fungi ~85%, Figure 2), while for others the offspring population was comparatively stable (e.g., wheat ~20% or lupine ~25%, Fig. 2). Also the dry weights (= body masses) of protonymphs (Fig. 3A, Tab. 3), deutonymphs (Fig. 3B, Tab. 3), tritonymphs (Fig. 3C, Tab. 3) and adults (Fig. 3D, Tab. 3) of *A. longisetosus* differed across all ten food resources (see Kruskal-Wallis tests in Tab. 3). While blood meal fed mites always were the lightest individuals (1.5–8.3 µg), specimens fed on yeast (8.3–24.5 µg) and on chlorella (6.8–28.1 µg) were the heaviest. Mites gained weight during their development, but the growth rate differed among resources (see slopes of body mass regressions in Tab. 3). Additionally, we found no relationship between mite density (Fig. 1, 2; Tab. 2) in the culture boxes and the offsprings' body mass (Fig. 3, Tab. 3) for any instar (OLS-regression: P_{Pro}= 0.16, P_{Deu}= 0.79, P_{Tri}= 0.96, P_{Adu}= 0.90).

3.3. Influence of nutrient quality on life history and reproductive traits

The numbers of individuals (= offspring) across all instars were always positively correlated to the C/N ratio in the food resources, but never to C/P or C/Ca (Tab. 4). The eclosion/hatching time was not correlated to any nutritional parameter, while the dry weights (= body masses) of the later instars of *A. longisetosus* (deuto-/

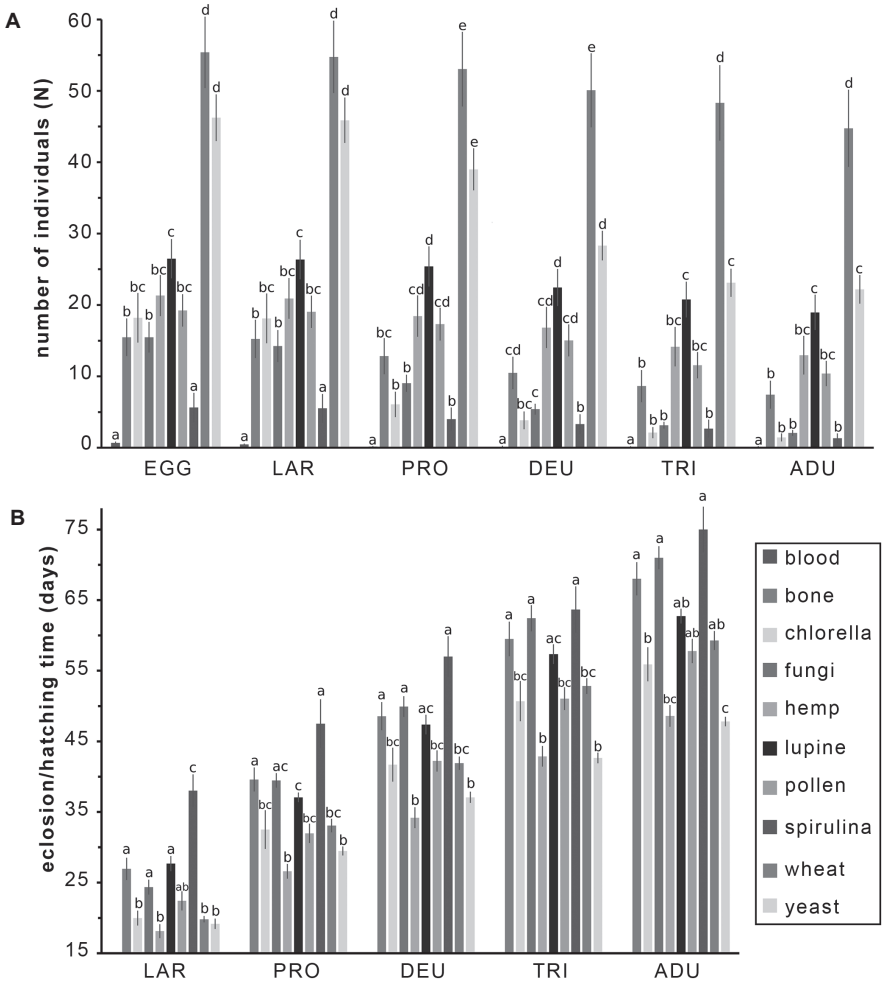


Figure 1. Number of individuals originated by one female (**A**) and eclosion/hatching time of her offspring, calculated as weighted mean (**B**) across the ten resources (see legend in **B**) and ontogenetic instars of *Archegozetes longisetosus*. Different letters indicate significant differences ($P < 0.05$) of pairwise Mann-Whitney-U tests within each instar. Bars represent means, error bars indicate standard errors. Blood meal was excluded in (**B**).

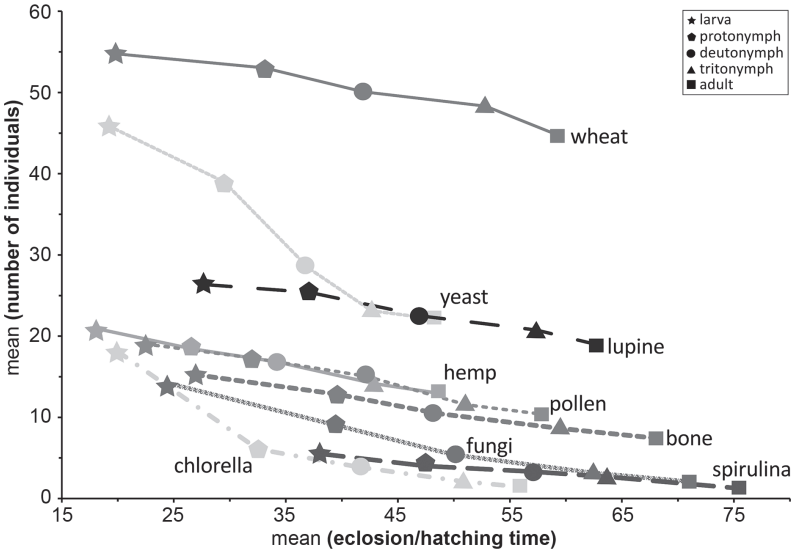


Figure 2. Survival curves of *Archegozetes longisetosus* cultured on nine different resources (blood meal was excluded) over a period of approximately 80 days.

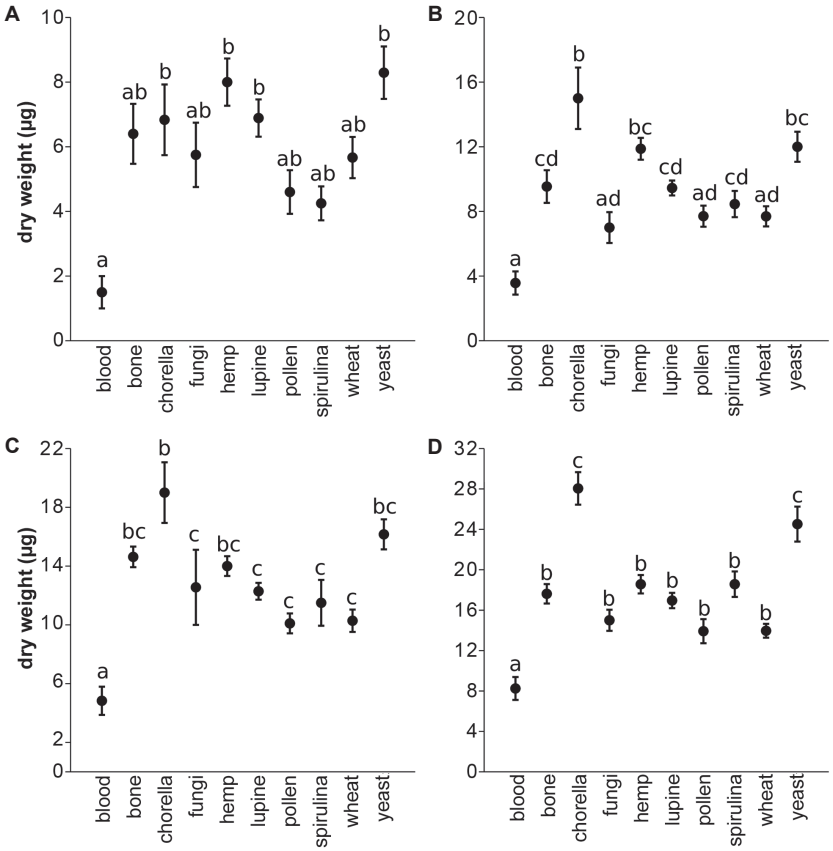


Figure 3. Mean dry weight of *Archegozetes longisetosus* proto- (**A**), deuto- (**B**), and tritonymphs (**C**), as well as adults (**D**), kept on different resources. Different letters indicate significant differences ($P < 0.05$) of pairwise Mann-Whitney-U tests within one ontogenetic instar. Circles represent means, error bars indicate standard errors.

Table 2. Life-history characteristics – the number of offspring per female (**A**) and the eclosion time (**B**) – of *Archegozetes longisetosus*. Reported numbers are means ± standard deviations. Friedman tests denote differences in the number of individuals within one food resource across all instars. Different letters indicate significant differences ($P < 0.05$) of pairwise Wilcoxon signed-rank tests across the instars of one resource (= post-hoc analyses of Friedman tests). *** = $P < 0.001$, NA = not applicable.

	egg	larva	protonymph	deutonymph	tritonymph	adult	
(A) NUMBERS							**Friedman test**
blood	0.68 ± 0.19	0.48 ± 0.11	0.16 ± 0.07	0.08 ± 0.05	0.08 ± 0.05	0.08 ± 0.05	NA
bone	15.48 ± 2.64a	15.24 ± 2.68a	12.84 ± 2.53b	10.48 ± 2.27c	8.64 ± 2.24d	7.44 ± 1.96e	$\chi^2_{5,143}$= 89.69 ***
chlorella	18.20 ± 3.47a	18.12 ± 3.48a	6.08 ± 1.78b	3.84 ± 1.25c	2.12 ± 0.78d	1.44 ± 0.56e	$\chi^2_{5,143}$= 106.68 ***
fungi	15.48 ± 2.14a	14.24 ± 2.24b	9.04 ± 1.20c	5.40 ± 0.76d	3.16 ± 0.48e	2.08 ± 0.42f	$\chi^2_{5,149}$= 100.42 ***
hemp	21.32 ± 2.88a	20.92 ± 2.85a	18.44 ± 2.90b	16.84 ± 2.87c	14.16 ± 2.76d	12.96 ± 2.71e	$\chi^2_{5,143}$= 108.44 ***
lupine	26.48 ± 2.74a	26.36 ± 2.78a	25.40 ± 2.81b	22.44 ± 2.60c	20.76 ± 2.50d	18.96 ± 2.48e	$\chi^2_{5,149}$= 74.12 ***
pollen	19.24 ± 2.28a	19.04 ± 2.26a	17.32 ± 2.31b	15.04 ± 2.24c	11.56 ± 1.84d	10.40 ± 1.77e	$\chi^2_{5,143}$= 90.32 ***
spirulina	5.64 ± 2.06a	5.52 ± 2.02a	4.00 ± 1.63b	3.32 ± 1.37c	2.68 ± 1.27d	1.32 ± 0.68e	$\chi^2_{5,65}$ = 34.88 ***
wheat	55.40 ± 5.00a	54.76 ± 5.06a	53.04 ± 5.23a	50.08 ± 5.19b	48.32 ± 5.30c	44.76 ± 5.41d	$\chi^2_{5,149}$= 59.49 ***
yeast	46.24 ± 3.27a	45.88 ± 3.18a	39.00 ± 2.95b	28.32 ± 2.07c	23.12 ± 1.97d	22.20 ± 2.01d	$\chi^2_{5,149}$= 121.67 ***
(B) ECLOSION TIME							
blood	-	20.93 ± 3.48	24.46 ± 4.00	26.00 ± 6.48	27.33 ± 4.24	31.00 ± 0.00	
bone	-	26.95 ± 1.58	39.60 ± 1.71	48.56 ± 2.00	59.51 ± 2.45	68.03 ± 2.35	
chlorella	-	19.99 ± 1.05	32.52 ± 2.73	41.69 ± 2.40	50.70 ± 2.85	55.91 ± 2.41	
fungi	-	24.36 ± 1.04	39.47 ± 1.02	49.92 ± 1.47	62.44 ± 1.85	71.00 ± 1.62	
hemp	-	18.13 ± 0.99	26.61 ± 1.04	34.19 ± 1.47	42.88 ± 1.46	48.61 ± 1.54	
lupine	-	27.69 ± 1.07	37.07 ± 0.69	47.38 ± 1.39	57.35 ± 1.39	62.74 ± 1.05	
pollen	-	22.42 ± 1.37	31.97 ± 1.38	42.24 ± 1.50	51.06 ± 1.62	57.80 ± 1.72	
spirulina	-	38.03 ± 2.30	47.51 ± 3.44	57.01 ± 2.87	63.66 ± 3.29	75.01 ± 3.24	
wheat	-	19.82 ± 0.46	33.08 ± 0.95	41.91 ± 0.93	52.83 ± 1.11	59.28 ± 1.34	
yeast	-	19.17 ± 0.73	29.48 ± 0.63	37.07 ± 0.80	42.66 ± 0.76	47.81 ± 0.68	

Table 3. Body mass of ontogenetic instars of *Archegozetes longisetosus*. Note that eggs and larvae were too small for individual weighing. Kruskal-Wallis tests denote differences among food resources within instars, while the body mass regression describe the growth of *A. longiseotsus* within one resource across the series of ontogenetic instars. Reported numbers are means ± standard deviations. Significant P-values are in **bold**; *** = P < 0.001, ** = P ≤ 0.01.

	protonymph	deutonymph	tritonymph	adult	body mass regression
blood	1.50 ± 0.50	3.57 ± 1.76	4.83 ± 3.18	8.25 ± 3.77	*slope*: 2.15, r^2 = 0.96, p = **0.018**
bone	6.40 ± 1.85	9.54 ± 3.50	14.63 ± 2.71	17.62 ± 4.29	*slope*: 3.88, r^2 = 0.99, p = **0.005**
chlorella	6.83 ± 3.62	15 ± 5.02	19.00 ± 6.84	28.05 ± 7.01	*slope*: 6.77, r^2 = 0.98, p = **0.009**
fungi	5.75 ± 2.63	7.00 ± 3.31	12.56 ± 7.23	15.00 ± 4.44	*slope*: 3.33, r^2 = 0.94, p = **0.027**
hemp	8.00 ± 2.19	11.88 ± 2.69	14.00 ± 2.77	18.57 ± 4.28	*slope*: 3.38, r^2 = 0.98, p = **0.009**
lupine	6.89 ± 2.38	9.45 ± 2.01	12.29 ± 2.57	16.95 ± 3.48	*slope*: 3.30, r^2 = 0.97, p = **0.011**
pollen	4.60 ± 2.52	7.71 ± 2.61	10.10 ± 2.95	13.92 ± 5.84	*slope*: 3.04, r^2 = 0.99, p = **0.004**
spirulina	4.25 ± 1.74	8.45 ± 2.57	11.50 ± 4.12	18.57 ± 4.55	*slope*: 4.60, r^2 = 0.97, p = **0.016**
wheat	5.67 ± 2.85	7.70 ± 2.93	10.28 ± 3.73	13.96 ± 3.35	*slope*: 2.75, r^2 = 0.98, p = **0.009**
yeast	8.29 ± 3.89	12.00 ± 4.55	16.16 ± 5.00	24.52 ± 8.49	*slope*: 5.29, r^2 = 0.95, p = **0.021**
	$\chi^2_{9,126}$ = 26.84 **	$\chi^2_{9,152}$ = 52.91 ***	$\chi^2_{9,156}$ = 61.38 ***	$\chi^2_{9,196}$ = 90.08 ***	

Table 4. Correlative relationships (ρS) of the nutritional composition (C/N, C/P, C/Ca) to the number of offspring, the eclosion times and dry weights of *Archegozetes longisetosus*. Significant P-values after false-discovery rate correction are denoted in **bold**.

	C/N	C/P	C/Ca
TOTAL NUMBER			
eggs	0.67, p = **0.032**	0.10, p = 0.79	-0.52, p = 0.13
larva	0.64, p = **0.047**	0.07, p = 0.85	-0.56, p = 0.09
protonymph	0.66, p = **0.038**	0.07, p = 0.82	-0.52, p = 0.13
deutonymph	0.66, p = **0.038**	0.08, p = 0.83	-0.51, p = 0.12
tritonymph	0.65, p = **0.043**	0.09, p = 0.80	-0.45, p = 0.19
adult	0.66, p = **0.037**	0.08, p = 0.83	-0.52, p = 0.13
ECLOSION TIME			
larva	-0.35, p = 0.36	0.02, p = 0.95	0.28, p = 0.46
protonymph	-0.42, p = 0.25	0.07, p = 0.84	0.12, p = 0.74
deutonymph	-0.20, p = 0.58	0.08, p = 0.81	0.40, p = 0.27
tritonymph	-0.25, p = 0.52	0.05, p = 0.88	0.28, p = 0.42
adult	-0.25, p = 0.51	0.05, p = 0.87	0.28, p = 0.46
DRY WEIGHT			
protonymph	0.16, p = 0.65	-0.53, p = 0.12	-0.65, p = **0.043**
deutonymph	-0.13, p = 0.73	-0.75, p = **0.013**	-0.63, p = **0.047**
tritonymph	-0.12, p = 0.72	-0.79, p = **0.006**	-0.59, p = 0.072
adult	-0.22, p = 0.53	-0.77, p = **0.009**	-0.36, p = 0.31

tritonymph and adults) were negatively correlated to C/P of the food (Tab. 4). Furthermore, the body masses of the earlier instars (proto-/deutonymphs) were negatively correlated to the C/Ca ratios (Tab. 4).

3.4. Reproductive output and developmental time

To investigate a possible trade-off between the number of offspring (= reproductive output) and the day until eclosion (= developmental time), we needed to transform both variables to project them on the same scale (see materials and methods). Both parameters were normalized as N' (output) and d' (speed). Thus, N' equals one for the sample replicate with the highest number of offspring and zero for the lowest number of offspring; reciprocally, d' equals one for the lowest developmental time and consequently lower d' represent faster development. Generally, we found no obvious trade-off between reproductive output and time (Fig. 4). *A. longisetosus* specimens fed on wheat grass had the highest N', but only an intermediate d'; on the other hand hemp and yeast cultured mites had low d'-values, yet only an intermediate-low reproductive output. The number of offspring N' in chlorella, fungi and spirulina treatments was very low, and the latter two also had the longest development d'.

4. Discussion

Overall, nutrient composition had an effect on the life history and reproductive biology of *A. longisetosus* across all instars (Fig. 1 and 2; Tab. 1–3), thus supporting the results of previous studies by Seniczak (1998) and Seniczak et al. (2016). Surprisingly, Estrada-Venegas et al. (1999) observed no effects of diet on life history of *A. longisetosus*, although dietary altered life histories are common among animals (e.g., Rushton & Hassall 1983, Boggs 1992, Elser et al. 1996, Jensen et al. 2011). Furthermore, several studies (Haq & Prabhoo 1977, Haq & Adolph 1981), suggested that *A. longisetosus* mainly feds on decomposed leaves, moss and certain soil fungi, but reject algae, yeast, lichens, seeds, vegetative tissue/bark of higher plants as well as food of animal origin in laboratory assays. Instead, our and others results (e.g., Honciuc 1996, Seniczak 1998, Smrz & Norton 2004, Seniczak 2006, Heethoff et al. 2007, Heidemann et al. 2011), clearly

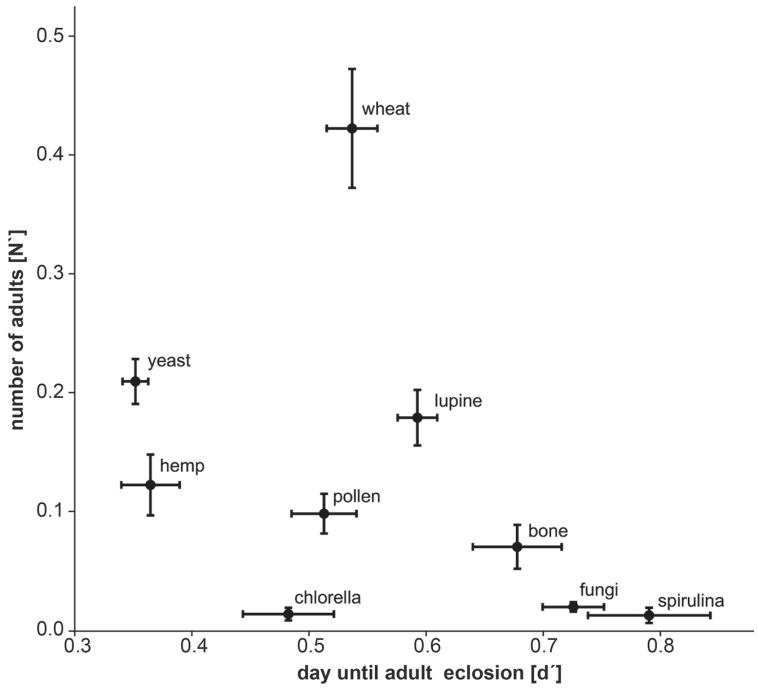


Figure 4. Comparison of the normalized number of adults (N'= reproductive output) and day until adult eclosion (d'= developmental time) of *Archegozetes longisetosus*. Higher N' and d' indicate more reproductive output and longer developmental time, respectively. Circles represent means, error bars indicate standard errors in both directions. Blood meals was excluded from this analysis.

demonstrate that *A. longisetosus* feeds and survives on various resources (Tab. 2) from different origins (animal, bacterial, fungal, herbal; Tab. 1). Hence, *A. longisetosus* may be classified as a broad opportunistic feeder (see also Heethoff & Scheu 2016), yet certain preferences for distinct resources, possibly related to olfactory signals of the food seem to be an innate characteristic of the mite (Brückner et al. 2018a, Brückner et al. 2018b). For instance, *A. longisetosus* appears to prefer food resources with a high amount of fat and thus uses fatty acids as a potential cue to find its food (Brückner et al. 2018a). We could demonstrate an adaptive value of this resource choice for chemical defense (i.e. food with high calories results in a faster regeneration of defensive secretions; Brückner & Heethoff 2018). However, there seems to be no connection of olfactory based food selection and life history, because *A. longisetosus* e.g. preferred lupine powder over wheat grass (Brückner et al. 2018b; this issue). Although *A. longisetosus* survived on every food we offered, there was a tremendous difference in all life history and reproductive traits across the resources. While some food led to high numbers of offspring (e.g. wheat, yeast, lupine, hemp or pollen), others just enabled the mites to survive (e.g., spirulina, chlorella and fungi) or were not able to foster stable reproduction (blood meal). The eclosion times and thus the developmental speed of the instars were strongly influenced by food, showing differences of about three (juvenile instars) to four (adults) weeks among the resources. This mechanism has also been studied in other arthropods groups (e.g., Barbosa & Capinera 1977, Gebhardt & Stearns 1988, Jiménez-Cortés et al. 2012), yet mostly the effects of diet are less pronounced compared to *A. longisetosus*. The strong influence of diet on body mass was expected, since animals generally show altered growth of their body (and fat storage) in response to dietary changes and composition (Case 1979, Demment & Van Soest 1985, Robinson & Redford 1986). While Seniczak (2006) found a density dependency of *A. longisetosus'* body size (smaller individuals at higher densities), we could not confirm this relationship in our experiments. In fact, we found that food indeed induced different densities (Fig. 1A), but the body mass never increased or decreased with lower or higher animal density across all investigated instars. The mites in our experiments had an unlimited access to food ('*ad libitum* conditions') and were hence not influenced by any nutritional shortages. Limited resource access, on the other hand, can lead to limited growth in animals as well as plants (e.g., Cohen 1971, Kozłowski 1992, Heino & Kaitala 1999) and might explain the effects observed in *A. longisetosus*. Since Seniczak (2006) did not clearly state whether the mites were cultured under *ad libitum* conditions or not, it is not possible to confirm or reject this idea.

In general, the number of offspring per female, as well as the size, but not the developmental time were influenced by macronutrients (C/N-, C/P- and C/Ca-ratios; Tab. 4). The correlation of the C/N-ratio – a proxy for nitrogen containing substances like amino acids – to the number of offspring of *A. longisetosus* is conclusive, because the availability of these substances is a prime regulator of growth and reproductive output in animals (e.g., Zanotto et al. 1993, Behmer et al. 2002, Fagan et al. 2002, Lee et al. 2003). As indicated by our data (Tab. 1 and 2), however, too high amounts of nitrogen (as indicated by a low C/N-ratio) as well as too low nitrogen (as indicated by a high C/N-ratio) are not optimal for high numbers of offspring. Hence, it seems evident that nitrogen requirements in *A. longisetosus* are not linear, but rather have an optimum – a phenomenon also quite common in the nutritional biology of other animals (e.g., Davis 1975, Ramsay & Houston 2003, Raubenheimer et al. 2005). A nitrogen optimum could also explain the results of Seniczak (1998) and Seniczak et al. (2016): tree bark and lichen protein contents were too low to yield in substantial offspring, *Protococcus* algae had an optimal amount of protein and could sustain the most offspring, while the protein content in napa cabbage appeared to be too high, yielding in a lower number of offspring again. As stated before, nutritional optima are quite common and can be explained by Bertrand's rule (Mertz 1981): at a low level of nutrients the benefits gained by more nutrients increase until a phase of equilibrium (= optimum), nutrients beyond are associated with increasing costs for the regulatory mechanisms yielding in disadvantage which are higher than the benefits gained by higher nutrient levels. While originally proposed for micronutrients, Raubenheimer et al. (2005) have shown that this principle also applies to macronutrients like carbohydrates, protein and P-content. Correspondingly, the same principle may not only apply to the C/N-ratio, but also to the correlation of C/P-ratio and body mass in *A. longisetosus*. However, the P-contents in the food resources we used were unevenly distributed and thus a potential P-optimum remains to be elucidated. Yet, we found that lower C/P-ratios (= higher P) had a positive effect on body mass; probably because nucleic acid and even more importantly ATP – the energy fueling the growth of all organisms – contains phosphorus (e.g., Call et al. 1978, Klausmeier et al. 2004). Interestingly, C/Ca-ratios were correlated to the body mass of earlier juvenile instars, which suggests that higher amounts of Ca are somehow important for the earlier life stages of *A. longisetosus*. Calcium-depended biomineralization (Norton & Behan-Pelletier 1991) would explain this pattern, however since *A. longisetosus* lacks this form of cuticular modification (Pachl et al. 2012), the positive influence of Ca on earlier instars remains unexplained. Developmental times in arthropods not only depend

on available nitrogen (Nestel et al. 2003), but also appear to be heavily influenced by the available energy of a food resource – stored as fat or carbohydrates (Florkin & Scheer 1970, Canavoso et al. 2001, Arrese & Soulages 2010). In invertebrates, a trade-off between the number and developmental time of offspring is common (Simmons 1987, Nunney 1996, Olofsson et al. 2009), however, we did not find that for *A. longisetosus* (Fig. 4). Instead, we found that certain resources (especially hemp, wheat, yeast) were able to sustain both – a relatively high reproductive output with a moderately long development time. This lack of constraints together with previously discussed results suggest that *A. longisetosus* requires resources of a distinct nutritional spectrum to sustainably stabilize its life history and reproductive energy budget. Thus, some resources – in our study for example blood meal or spirulina – may have a too imbalanced nutritional composition, yielding low reproductive rates, long developmental times and smaller offspring.

5. Acknowledgments

We thank Andrea Hilpert, Ursula Lebong, Christian Storm, Sonja Elberich, Danny Rothe and Tim Bergmann for experimental assistance. We are also grateful to Nico Blüthgen for statistical advice and Roy A. Norton for his long-lasting support. AB was supported by a PhD scholarship of the German Nation Academic Foundation (Studienstiftung des deutschen Volkes). This study was funded by the German Science Foundation (DFG; HE 4593/5-1).

6. Authors' contributions

AB and MH designed the research; AB, RS and KW performed the experiment, AB performed chemical analyses; AB analyzed the data; AB and MH wrote the paper. All authors discussed and approved the final manuscript.

7. References

Aoki, J. (1965): Oribatiden (Acarina) Thailands. I. – Nature and Life in Southeast Asia **4**:129–193.

Arrese, E. L. & J. L. Soulages (2010): Insect fat body: energy, metabolism, and regulation. – Annual Review of Entomology **55**: 207–225.

Barbosa, P. & J. L. Capinera (1977): The influence of food on developmental characteristics of the gypsy moth, *Lymantria dispar* (L.). – Canadian Journal of Zoology **55**: 1424–1429.

Bastida, F., E. Kandeler, J. L. Moreno, M. Ros, C. Garcia & T. Hernandez (2008): Application of fresh and composted organic wastes modifies structure, size and activity of soil microbial community under semiarid climate. – Applied Soil Ecology **40**: 318–329.

Beck, L. (1967): Beiträge zur Kenntnis der neotropischen Oribatidenfauna. 5. *Archegozetes* (Arach., Acari). – Senckenberg Biologie **48**: 407–414.

Behmer, S. T., S. J. Simpson & D. Raubenheimer (2002): Herbivore foraging in chemically heterogeneous environments: nutrients and secondary metabolites. – Ecology **83**: 2489–2501.

Benjamini, Y. & Y. Hochberg (1995): Controlling the false discovery rate - a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B-Methodological **57**: 289–300.

Boggs, C. (1992): Resource allocation: exploring connections between foraging and life history. – Functional Ecology **6**: 508–518.

Borken, W., A. Muhs & F. Beese (2002): Application of compost in spruce forests: effects on soil respiration, basal respiration and microbial biomass. – Forest Ecology and Management **159**: 49–58.

Brückner, A. & M. Heethoff (2018): Nutritional effects on chemical defense alter predator–prey dynamics. – Chemoecology **28** [doi.org/10.1007/s00049-018-0253-9].

Brückner, A., R. Schuster, T. Smit & M. Heethoff (2018b). Imprinted or innated food preferences in the model mite *Archegozetes longisetosus* (Actinotrichida, Oribatida, Trhypochthoniidae). – Soil Organisms **90** (1): 23–26.

Brückner, A., R. Schuster, T. Smit, M. M. Pollierer, I. Schäffler & M. Heethoff (2018a): Track the snack – Olfactory cues shape foraging behaviour of decomposing soil mites (Oribatida). Pedobiologia **66:** 74–80.

Call, J., J. Butcher, J. Blake, R. Smart & J. Shupe. (1978): Phosphorus influence on growth and reproduction of beef cattle. – Journal of Animal Science **47**: 216–225.

Canavoso, L. E., Z. E. Jouni, K. J. Karnas, J. E. Pennington & M. A. Wells (2001): Fat metabolism in insects. – Annual Review of Nutrition **21**: 23–46.

Case, T. J. (1979): Optimal body size and an animal's diet. – Acta Biotheoretica **28**: 54–69.

Cohen, D. (1971): Maximizing final yield when growth is limited by time or by limiting resources. – Journal of Theoretical Biology **33**: 299–307.

Davis, G. (1975): Essential dietary amino acids for growth of larvae of the yellow mealworm, *Tenebrio molitor* L. – The Journal of Nutrition **105**: 1071–1075.

Demment, M. W. & P. J. Van Soest (1985): A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. – American Naturalist **125**: 641–672.

Elser, J. J., D. R. Dobberfuhl, N. A. MacKay & J. H. Schampel (1996): Organism size, life history, and N: P stoichiometry. – BioScience **46**: 674–684.

Estrada-Venegas, E. G., R. A. Norton, A. Equihua-Martínez, R. Nápoles, J. Trinidad Santos & H. González Hernandez (1999): Biologia y nueva sinonima de *Archegozetes longisetosus* Aoki (Acari - Oribatida) de la Mancha, Veracruz, Mexico. – Folia Entomologica Mexico **107**: 41–50.

Fagan, W. F., E. Siemann, C. Mitter, R. F. Denno, A. F. Huberty, H. A. Woods & J. J. Elser (2002): Nitrogen in insects: implications for trophic complexity and species diversification. – American Naturalist **160**: 784–802.

Florkin, M. & B. Scheer (1970): Chemical Zoology. – Academic Press, New York.

Friedman, M. (1937): The Use of Ranks to Avoid the Assumption of Normality Implicit in the Analysis of Variance. – Journal of the American Statistical Association **32**: 675–701.

Gebhardt, M. D. & S. C. Stearns (1988): Reaction norms for developmental time and weight at eclosion in *Drosophila mercatorum*. – Journal of Evolutionary Biology **1**: 335–354.

Hammer, Ø., D. A. T. Harper & P. D. Ryan (2001): PAST: Paleontological statistics software package for education and data analysis. – Palaeontologia Electronica **4**: 9.

Haq, M. A. (1978): Breeding Biology of Oribatid Mites. – In: Edwards C. A. & G. K. Veesesh (eds): Soil Biology and Ecology in India. – Hebbol, Bangalore: 145–151.

Haq, M. A. (1982): Pheromonal regulation of aggregation and moulting in *Archegozetes longisetosus* (Acari: Oribatei). Proceedings of the 11th Annual Conference of the Ethological Society of India. – Calicut University Research Journal, Calicut: 19.

Haq, M. A. & C. Adolph (1981): A comparative study of the duration of the life cycles of four species of oribatid mites (Acari: Oribatida) from the soils of Kerala. Indian Journal of Acarology **5**: 56–61.

Haq, M. A. & N. R. Prabhoo (1977): Observations on the feeding habits of oribatid mites from the soils of Kerala (Acarina: Cryptostigmata). – Entomon **1**: 133–137.

Heethoff, M., M. Laumann & P. Bergmann (2007): Adding to the reproductive biology of the parthenogenetic oribatid mite, *Archegozetes longisetosus* (Acari, Oribatida, Trhypochthoniidae). – Turkish Journal of Zoology **31**: 151–159.

Heethoff, M. & S. Scheu (2016): Reliability of isotopic fractionation (Δ15N, Δ13C) for the delimitation of trophic levels of oribatid mites: Diet strongly affects Δ13C but not Δ15N. – Soil Biology & Biochemistry **101**: 124–129.

Heethoff, M., P. Bergmann, M. Laumann & R. A. Norton. (2013): The 20th anniversary of a model mite: A review of current knowledge about *Archegozetes longisetosus* (Acari, Oribatida). – Acarologia **53**: 353–368.

Heidemann, K., S. Scheu, L. Ruess & M. Maraun (2011): Molecular detection of nematode predation and scavenging in oribatid mites: Laboratory and field experiments. – Soil Biology & Biochemistry **43**: 229–236.

Heino, M. & V. Kaitala (1999): Evolution of resource allocation between growth and reproduction in animals with indeterminate growth. – Journal of Evolutionary Biology **12**: 423–429.

Honciuc, V. (1996): Laboratory studies of the behavior and life cycle of *Archegozetes longisetosus* Aoki 1965 (Oribatida). In Acarology IX. Proceedings. – Ohio Biological Survey, Columbus: 637–640.

Jensen, K., D. Mayntz, S. Toft, D. Raubenheimer & S. J. Simpson (2011): Prey nutrient composition has different effects on *Pardosa* wolf spiders with dissimilar life histories. – Oecologia **165**: 577–583.

Jiménez-Cortés, J. G., M. A. Serrano-Meneses & A. Córdoba-Aguilar (2012): The effects of food shortage during larval development on adult body size, body mass, physiology and developmental time in a tropical damselfly. – Journal of Insect Physiology **58**: 318–326.

Klausmeier, C. A., E. Litchman, T. Daufresne & S. A. Levin. (2004): Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. – Nature **429**: 171–174.

Kozłowski, J. (1992): Optimal allocation of resources to growth and reproduction: implications for age and size at maturity. – Trends in Ecology & Evolution **7**: 15–19.

Kruskal, W. H. & W. A. Wallis (1952): Use of ranks in one-criterion variance analysis. – Journal of the American Statistical Association **47**: 583–621.

Lee, K. P., D. Raubenheimer, S. T. Behmer & S. J. Simpson (2003): A correlation between macronutrient balancing and insect host-plant range: evidence from the specialist caterpillar *Spodoptera exempta* (Walker). – Journal of Insect Physiology **49**: 1161–1171.

Mann, H. B. & D. R. Whitney (1947): On a test of whether one of two random variables is stochastically larger than the other. – Annals of Mathematical Statistics **18**: 50–60.

Mertz, W. (1981): The essential trace elements. – Science **213**: 1332–1338.

Nestel, D., D. Tolmasky, A. Rabossi & L. A. Quesada-Allué. (2003): Lipid, carbohydrates and protein patterns during metamorphosis of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). – Annals of the Entomological Society of America **96**: 237–244.

Norton, R. A. & V. M. Behan-Pelletier (1991): Calcium carbonate and calcium oxalate as cuticular hardening agents in oribatid mites (Acari: Oribatida). – Canadian Journal of Zoology **69**: 1504–1511.

Nunney, L. (1996): The response to selection for fast larval development in *Drosophila melanogaster* and its effect on adult weight: an example of a fitness trade-off. – Evolution **50**: 1193–1204.

Olofsson, H., J. Ripa & N. Jonzén (2009): Bet-hedging as an evolutionary game: the trade-off between egg size and

number. – Proceedings of the Royal Society of London B: – Biological Sciences **276**: 2963–2969.

Pachl, P., K. Domes, G. Schulz, R. A. Norton, S. Scheu, I. Schaefer & M. Maraun (2012): Convergent evolution of defense mechanisms in oribatid mites (Acari, Oribatida) shows no "ghosts of predation past". – Molecular Phylogenetics and Evolution **65**: 412–420.

R Core Team (2016): R: A language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, Austria [http://www.R-project.org].

Ramsay, S. L. & D. C. Houston (2003): Amino acid composition of some woodland arthropods and its implications for breeding tits and other passerines. – Ibis **145**: 227–232.

Raubenheimer, D., K. Lee & S. Simpson (2005): Does Bertrand's rule apply to macronutrients? – Proceedings of the Royal Society of London B: Biological Sciences **272**: 2429–2434.

Robinson, J. G. & K. H. Redford (1986): Body size, diet, and population density of Neotropical forest mammals. American Naturalist **128**: 665–680.

Rushton, S. P. & M. Hassall (1983): The effects of food quality on the life history parameters of the terrestrial isopod (*Armadillidium vulgare* (Latreille)). – Oecologia **57**: 257–261.

Seniczak, A. (1998): Preliminary studies on the influence of food on the development and morphology of *Archegozetes longisetosus* Aoki (Acari, Oribatida) in laboratory conditions. – Ochrona Srodowiska (Poland) **2**: 175–180.

Seniczak, A. (2006): The effect of density on life-history parameters and morphology of *Archegozetes longisetosus* Aoki, 1965 (Acari: Oribatida) in laboratory conditions. – Biological Letters **43**: 209–213.

Seniczak, A., A. Ligocka, S. Seniczak & Z. Paluszak (2016): Effects of green algae and napa cabbage on life-history parameters and gut microflora of *Archegozetes longisetosus* (Acari: Oribatida) under laboratory conditions. – Biological Letters **53**: 67–78.

Simmons, L. (1987): Female choice contributes to offspring fitness in the field cricket, *Gryllus bimaculatus* (De Geer). – Behavioral Ecology and Sociobiology **21**: 313–321.

Smrz, J. & R. A. Norton (2004): Food selection and internal processing in *Archegozetes longisetosus* (Acari: Oribatida). – Pedobiologia **48**: 111–120.

Spearman, C. (1904): "General Intelligence", objectively determined and measured. – The American Journal of Psychology **15**: 201–292.

Subias, L. S. (2004): Listado sistemático, sinonímico y biogeográfico de los ácaros oribátidos (Acariformes, Oribatida) del mundo. – Graellisa **60**: 3–305.

Wilcoxon, F. (1945): Individual comparisons by ranking methods. – Biometrics Bulletin **1**: 80–83.

Zanotto, F., S. Simpson & D. Raubenheimer (1993): The regulation of growth by locusts through post-ingestive compensation for variation in the levels of dietary protein and carbohydrate. – Physiological Entomology **18**: 425–434.

10.1.2 Publication 2: **Nutritional quality modulates intraspecific trait variability**

Adrian Brückner, Romina Schuster, Katja Wehner and Michael Heethoff

Submitted to Frontiers in Zoology

Submitted: 02.03.2018

Authors contributions:

AB and MH designed the research; AB, RS and KW performed the experiment, AB performed chemical analyses; AB analyzed the data; AB and MH wrote the paper. All authors discussed and approved the final manuscript.

Brückner Adrian, Schuster Romina, Wehner Katja, Heethoff Michael. © The authors 2018

Nutritional quality modulates intraspecific trait variability

Adrian Brückner[1,*] Romina Schuster[1], Katja Wehner[1] and Michael Heethoff[1,*]

[1]*Ecological Networks, Technische Univeristät Darmstadt, Schnittspahnstraße 3, 64287 Darmstadt, Germany*

**Corresponding authors: heethoff@bio.tu-darmstadt.de, adrian.brueckner@gmail.com*

Abstract

Background: Trait based functional and community ecology is *en vogue*. Most studies, however, ignore phenotypical diversity by characterizing entire species considering only trait means rather than their variability. Phenotypical variability may arise from genotypical differences or from ecological factors (e.g., nutritionally imbalanced diet), and these causes can usually not be separated in natural populations. We used a parthenogenetic model system (the oribatid mite *Archegozetes longisetosus* Aoki) to exclude genotypical differences and quantify exclusively ecologically induced trait variation. We investigated patterns of dietary (10 different food treatments) induced trait variation by measuring the response of nine different traits (from life history, morphology and exocrine gland chemistry).

Results: Nutritional quality (measured as carbon-to-nitrogen ratio) influenced all trait means and their variation. Some traits were more prone to variation than others. Furthermore, the "threshold elemental ratio" (for element stoichiometry) or "Bertrand's rule" (for marcro-/micronutrients) applied to phenotypic trait variation. Imbalanced food led to lower trait mean values, but also to a higher variation of traits.

Conclusion: The *variation of trait means* seems to be a predictor for food quality, while the *variation of trait variation* (across many traits of animals fed on one resources) may be an indicator for trade-offs an animal has to deal with while feeding on a particular diet.

Keywords

trait plasticity, functional traits, parthenogenesis, Bertrand´s rule, nutritional balance, threshold elemental ratio, oribatid mites, *Archegozetes longisetosus*

1. Background

The theory of phenotypic plasticity predicts that virtually all biological systems inherently include variation of characters at all organization levels [1-3]. This variation can be induced by virtually any external factor, leading to a nearly infinite number of possible phenotypic specificities [4, 5]. Phenotypic plasticity can therefore be defined as the degree of a single genotype to express variation in multiple traits leading to different phenotypes in response to environmental gradients [3, 6]. Trait based approaches are *en vogue* in all fields of ecology [7, 8], yet it has been assumed that trait means of a species can be used to sufficiently characterize communities and affiliated functionality [9, 10]. Natural populations, however, consists of phenotypically diverse individuals, possessing variable traits [3, 11, 12]. Understanding causes and mechanism leading to trait variation within populations can thus give unprecedented resolution to explain evolutionary ecology dynamics underpinning community structure, trait assemblages, and stability [e.g., 11, 13, 14].

Theory [15], but also some experimental evidence [e.g., 16, 17] suggest that phenotypic plasticity may arises from trade-offs among multiple traits within a species that are ultimately caused by imbalances in elemental stoichiometry or nutritional components of food. For instance, nutrients influence fitness related traits [e.g., 18, 19-21], but also body shapes [22], or intraspecific chemical communication [e.g. 23] of an animal, and may even alter biological rules [24]. In this context, a resource can be considered optimal if i) it enables an animal to respond with the highest possible trait mean, but also ii) can sustain phenotypes with low trait variability and thus a stable performance [see 18, 20, 25]. Yet, reaction norms

of only a limited number of distinct traits towards a broad gradient of macronutrients [20] or elements [26] have been described and the relationship of variation and elemental balance or macronutrients across multiple traits (i.e. phenotypic plasticity) is, to our best knowledge, still unknown.

Here, we harness the power of a unique model system – the all-female parthenogenetic oribatid mite *Archegozetes longisetosus* Aoki, which reproduces *via* automixis with an inverted meiosis resulting in quasi-clonal offspring [27, 28]. While heritable genetic variation of traits cannot clearly be distinguished from environmentally induced trait variation (phenotypic plasticity) in sexual species, clonal systems provide the possibility to quantify reaction norms of traits and their variation [e.g., 29, 30-32]. We selected a variety of different individual and demographic life-history, morphological and defensive traits to quantify their response to food with different nutritional quality. We fed ten resources, differing in C/N ratios (as well as macro- and micronutrients; see materials and methods), to the mites, and measured the mean and variation of traits along this food quality gradient. Thus, we exploit our "phenotypically-tractable" model species (i.e. a model system without genotypic, but only phenotypic variation), to decipher patterns and mechanisms of dietary induced trait plasticity. More specifically we ask how nutritional quality (measured as C/N ratios) influences the expression of multiple traits regarding i) trait means, ii) trait variation, and iii) differences among traits.

2. Materials and Methods

2.1 Experimental setup and trait selection

Archegozetes longesitosus ran [27] were reared at approx. 28°C and 80-85% relative humidity in constant darkness on one out of ten resources for several generations (approx. 18 month). All specimens of this strain originate from a single gravid female collected in Puerto

72 Rico in 1993. Due to the quasi-clonal reproduction, all mites used for the experiments are
73 genotypically identical [27]. The ten resources were blood meal (blood; Common Baits,
74 Rosenfeld, Germany), bone meal (bone; Canina Pharma GmbH, Hamm, Germany), *Spirulina*
75 powder (spirulina; Interaquaristik, Biedenkopf-Breidenstein, Germany), shiitake fungus
76 powder (fungi; Arche Naturprodukte GmbH, Hilden, Germany), grinded dry yeast (yeast;
77 Rapunzel Naturkost GmbH, Legau, Germany), *Chlorella* powder (chlorella; Naturya, Bath,
78 UK), hemp protein powder (hemp; Naturya, Bath, UK), sweet lupine flour (lupine; Govinda
79 Natur GmbH, Neuhofen, Germany), grinded mixed pollen grain (pollen; Ascopharm GmbH,
80 Wernigerode, Germany) and wheat grass powder (wheat; Naturya, Bath, UK). Fresh food and
81 water was provided *ad libitum* three times a week. For each resource, specimens were
82 cultured in three separated plastic boxes (100x100x50 mm) grounded with 2 cm mixture of
83 plaster of Paris/activated charcoal mixture (9:1).

84 As traits we selected life-history, morphological and chemical characters (Fig. 1, Table
85 1). The life-history traits were selected to quantify the reproductive fitness of one female
86 based on its offspring [30, 32, 33], while morphological traits were used to describe changes
87 in size and body shape [2]. All chemical traits were related to defensive exocrine opisthonotal
88 oil-glands [34, 35]. Oil-gland chemistry plays an important role in structuring feeding
89 interactions in soil food webs and enables oribatid mites to live in “enemy-free space” [36,
90 37], i.e. a conceptual way of living that reduces or eliminate a species' vulnerability against
91 their predators [38].

92 At the start of the experiment we selected young (approximately one week after
93 eclosing) adult individuals from their original culture-plates. We directly used 130 specimens
94 per resource (= 1300 in total) for chemical experiments, while 25 specimens per resource (=
95 250 in total) were individually redistributed into smaller culture boxes (45 x 40 x 35 mm;
96 grounded with the plaster of Paris mixture) for further experiments.

97 2.2.1 Chemical experiments and analysis

98 Oil-gland extractions of 30 specimens from each of the resources (n=10*30) were
99 accomplished by immersing individuals in 50 µl hexane (GC grade, 98% purity purchased
100 from Merck, Darmstadt, Germany) for 3 minutes (see Fig. 1). The extraction solvent also
101 contained tetradecane (1 ng/µl; ≥99.8%, analytical standard, purchased from Sigma-Aldrich,
102 Munich, Germany) as an internal standard. Crude extracts were used for gas chromatography-
103 mass spectrometry (GC/MS) analyses and mite specimens were stored at -20°C for further
104 measurements. Samples were analyzed with a QP 2010ultra GC-MS (Shimadzu, Duisburg,
105 Germany) according to a protocol given elsewhere [39]. The gas chromatograph was
106 equipped with a ZB-5MS fused silica capillary column (30 m x 0.25 mm ID, df= 0.25 µm)
107 from Phenomenex (Aschaffenburg, Germany). The extracted mites were dried at 60°C until
108 weight constancy and the dry weight was determined with a microbalance (Mettler Toledo,
109 XS3DU, 0.1 µg readability and 1 µg repeatability) to calculate the secretion amount per
110 animal/dry weight in [ng/µg], see [39]. Furthermore, we used the chemical data to quantify
111 the relative amounts in [%] of all oil gland compounds based on peak areas. All compounds
112 have been identified previously [for details see 39], and were assigned using their retention
113 indices and diagnostic ions.

114 Additionally, 100 specimens per resources were artificially disarmed using an
115 established hexane-recovery-hexane (HRH) protocol which does not influence the mites' life-
116 history, but results in the complete depletion of the glands [33; see Fig. 1]. Mites were
117 redistributed to culture plates (n=10 plates per resource, with 10 individuals per plate resulting
118 in 100 samples) and fed with the same resources mentioned above. After 28 days, specimens
119 were extracted in hexane to measure the proportion of specimens that have regenerated of oil-
120 gland secretions [see 40; Table 1].

121 2.2.2 Life history experiments

122 Singularized mites (= mothers, n=10*25) were allowed to lay eggs for ten days and the
123 same food and water was provided *ad libitum* three times a week (see Fig. 1). Every box was
124 checked daily, and we counted the number all eggs, juvenile instars (i.e. larva, protonymph,
125 deutonymph, tritonymph) and adults for a period of up to 12 weeks. We removed freshly
126 hatched adults from the culture plates to ensure no new egg deposition and froze them for
127 further analysis. The total developmental time [days] for each mother's offspring was
128 calculated as weighted arithmetic mean (developmental time= $\sum [d_i * p_i]$; where d_i is the
129 experiment day and p_i is the proportion of new adult specimens on d_i). Replicates were all
130 offspring died before reaching adulthood were not used to calculate the developmental time
131 (n= 72). In addition to the counted data (see Table 1) we dried all individuals at 60°C to
132 determine the total biomass output of individual mothers, expressed as dry weight [mg] per
133 female.

134 2.2.3 Morphological analysis

135 The removed mothers from the life-history experiments were used for morphological
136 analysis (see Fig. 1, Table 1), except for damaged individuals that were excluded from the
137 measurements. We overall measured eleven continuous variables of individual specimen (five
138 dorsal and six ventral distances) for the morphometric analysis (see supplementary Fig.S1 for
139 details) using a VHX-5000 microscope (Keyence Deutschland GmbH, Neu-Isenburg,
140 Germany) equipped with the VH-Z50L lens. To remove the effect of isometric body size
141 scaling on morphometrical measures we standardized all values of one individual by its
142 notogaster length (see supplemental information). All measured individuals were additionally
143 dried at 60°C and the body mass, expressed as dry weight [µg] was determined as previously
144 described.

145 2.2.4 Analyses of nutritional quality

146 For C/N analyses dried resource powders (5±1 mg) were weighed into tin capsules.
147 Total organic carbon and nitrogen contents were measured by an elemental analyzer (EA
148 1108 Elemental Analyser, Carlo Erba, Milan, Italy). Acetanilide (Merck, Darmstadt,
149 Germany) was used as standard. Carbon and nitrogen amounts were calculated based on the
150 standard and the initial dry weight and expressed as C/N ratios (Table 2).

151 2.3 Data analysis

152 We analyzed the univariate traits (see Table 1) using Kruskal-Wallis tests and Levene
153 tests to access the overall differences and the variance among resources, respectively. Posthoc
154 comparisons using Dunn's test [41] and false discovery rate [42] to correct for multiple test
155 can be found in the supplementary material S2. For multivariate traits [=chemical
156 composition (as Bray-Curtis similarities) and morphometric measures (as Euclidean
157 distances)] we also analyzed the differences and variances among resources using
158 PERMANOVA [43] and PERMDISP [43], respectively. Both multivariate traits were
159 ordinated using discriminant analysis of principal components [DAPC; see 44]. DAPC
160 transforms the original data by principal component analysis (PCA) prior to the discriminant
161 analysis. We retained 6 (for chemical composition) and 7 (for morphometry) PC-axes based
162 on their Eigenvalues and the explained variance.

163 We visualize the response of a certain trait to the resource quality parameter (i.e. C/N-
164 ratio) with the generic nls-function in R and fitted either a linear response ($y \sim bx$) or an
165 optimum curve ($y \sim cx^2 + dx$; where "c" is the curvature), based on lower AIC [45]. To check
166 for significance of the linear or optimum response curve, we tested if the slope "b" or the
167 curvature "c" significantly differed from 0 [2].

168 To quantify the trait (Table 1) variation we calculated the coefficient of variation - CV
169 % = (standard deviation/ mean *100) - for all ten resource treatments and every trait, yielding
170 in nine different trait CVs for each resource. To summarize the multivariate traits (chemical

171 composition and morphometry) we first calculated the CVs for each single component and
172 subsequently averaged the single CVs to obtain the mean CV for both traits. Afterwards, we
173 used these values to calculate the mean CV for each resource and correlated these averaged
174 CVs with the C/N-ratios using Spearman´s rank (see last line in Table 3). We used a Kruskal-
175 Wallis test to check whether the nine trait CVs across all resources are prone to more/less
176 variation compared to others and if trait types (chemical, life history, morphology) possess
177 different variability using the mean across resource CVs. Additionally, we analyzed the mean
178 trait variation across traits to test whether some resources produce more variable traits than
179 others and if the variation of trait variation differs across resources using a Kruskal-Wallis
180 and Levene test, respectively.

181 The following replicates were not included in the statistical analyses: chemical
182 samples with contaminations for secretion amounts (n= 14); mothers which did not survive
183 the egg laying period for life history data (n= 34); total biomass output lower than 1 µg (not
184 reliably measurable, n= 8); non intact mothers from mass measurement (n= 8); damaged
185 specimen for morphometric measures (n= 47). The raw data is deposited in supplementary
186 material S3. All statistical analyses were performed with R 3.3.2 [46], using the packages
187 “adegenet” [44], “car” [47], “PMCMR” [48], “vegan”, “ and “Rcmdr” [49].

188 ## 3. Results

189 3.1 Nutritional quality of the food

190 Some food resources were characterized by an extreme composition – e.g. blood meal
191 had a very high amount nitrogen (13.3±0.1% N; mean±SD), and pollen was carbon rich
192 (3.6±0.1% N; mean±SD), but most of the remaining resources were quite balanced (Table 2).
193 Thus, the variability of the C/N ratios across the food was high (CV= 47%; 7.5±3.5;
194 mean±SD), and the spanned from 3.3 (blood meal) to 13.6 (pollen).

195 3.2 Chemical traits

196 Three traits were related to chemical defense (Table 1; Fig. 2). The individual amount
197 of defensive secretions (ng/µg; Kruskal-Wallis: n= 286, df= 9, χ^2= 62.74, p< 0.0001; Fig. 2a;
198 Table 3) and its variation within each group (Levene: $F_{9,276}$= 11.25, p< 0.0001; Fig. 2a; Table
199 3) differed across all resources. The individual secretion amount showed an optimum for the
200 C/N ratio (curvature: -0.081±0.019, t= 4.2, p< 0.001; ΔAIC=15.1; Fig. 2b). The fraction of
201 regenerating individuals (%; Kruskal-Wallis: N= 100, df= 9, χ^2= 42.97, P< 0.0001; Fig. 2c;
202 Table 3) and their variation within each group (Levene: $F_{9,90}$= 2.99, P= 0.004; Fig. 2c; Table
203 3) differed across all resources. The fraction of regenerating individuals again showed an
204 optimum for the C/N ratio (curvature: -1.298±0.353, t= 3.7, p< 0.001; ΔAIC=11.1; Fig. 2d).
205 Also, the relative composition (%;Fig. 2e; Table 3) of the seven compounds found in the
206 defensive secretions showed differences among groups (PERMANOVA: pseudo-$F_{9,276}$=
207 14.01, r^2= 0.31, p< 0.0001) and in multivariate dispersion (=variation; PERMDISP: $F_{9,276}$=
208 3.91, p< 0.001; see group spreads in Fig. 2e).

209 3.3 Life-history traits

210 In total, we investigated four life-history traits, related to reproductive fitness and
211 resource allocation (Table 1, Fig. 3). The developmental time of each females' offspring,
212 calculated as weighted mean (days; Kruskal-Wallis: n= 172, df= 8, χ^2= 100.54, p< 0.0001;
213 Fig. 3a; Table 3) and its variation within each group (Levene: $F_{8,163}$= 3.22, p= 0.002; Fig. 3a;
214 Table 3) differed across all resources. Blood meal was excluded from the analyses, because
215 only one individual developed from egg to adult. The survival of each females' offspring (%;
216 n= 216, df= 9, χ^2= 112.47, p< 0.0001; Fig. 2c; Table 3) and its variation within each group
217 (Levene: $F_{9,206}$= 4.02, p< 0.0001; Fig. 2c; Table 3) differed across all resources. The total
218 number of offspring per female (N*female^{-1}; Kruskal-Wallis: n= 216, df= 9, χ 2= 134.80, p<
219 0.0001; Fig. 3e; Table 3) and its variation within each group (Levene: $F_{9,206}$= 7.97, p< 0.0001;
220 Fig. 3e; Table 3) differed significantly across all resources. The reproductive output per

female (mg*female^{-1}; Kruskal-Wallis: n= 208, df= 9, χ^2= 125.19, p< 0.0001; Fig. 3g; Table 3) and its variation within each group (Levene: $F_{9,198}$= 5.04, p< 0.0001; Fig. 3g; Table 3) also differed across all resources. All life-history traits responded with optima curves (Fig. 3b,d,f,h) to the C/N ratio gradient (developmental time. curvature: 0.326±0.108, t= 3.0, p= 0.003; ΔAIC=7.1; Fig. 3b: survival of one females' offspring; curvature: -0.584± 0.257, t= 2.3, p= 0.024; ΔAIC=3.2; Fig. 3d: total number of offspring per female; curvature: -0.373±0.138, t= 2.7, p= 0.007; ΔAIC=5.3; Fig. 3f: reproductive output per female; curvature: -27.57±7.20, t= 3.8, p< 0.001; ΔAIC=12.4; Fig. 3h).

3.4 Morphological traits

Body mass and various morphometric measurements describing body shape were included (Table 1, Fig. 4). The individual body masses (µg; Kruskal-Wallis: n= 208, df= 9, χ^2= 89.06, p< 0.0001; Fig. 4a; Table 3) and their variation within each group (Levene: $F_{9,198}$= 2.60, p= 0.007; Fig. 4a; Table 3) differed across all resources and again followed a C/N optimum (curvature: -0.337±0.055, t= 6.2, p< 0.001; ΔAIC=33.4; Fig. 4b). The eleven morphometric characters (% NL; Table 3; see supplement for character overview) measured for individual mites showed moderate, yet differences among groups (PERMANOVA: pseudo-$F_{9,192}$= 2.36, r^2= 0.10, p= 0.003; Fig. 4c), but no differences in multivariate dispersion (=variation; $F_{9,192}$= 1.51, p=0.147; see equal group spreads in Fig. 4c).

3.5 Trait variation

All traits (Table 1) were influenced by diet (Fig.s 2-4; Table 3) and varied (calculated as CV [%]) across all resources (Table 3). Also, CVs of the traits (Table 1) differed (Kruskal-Wallis: n= 89, df= 8, χ^2= 61.75, p< 0.0001; Fig. 5a). Generally, the variability was the same among the trait types (Kruskal-Wallis: n= 9, df= 2, χ^2= 1.41, p= 0.49). Life-history had a CV_{mean}= 62% (CVs for dev. time, survival, offspring and output were 11%, 45%, 125% and 112%, respectively), chemical traits responded with a CV_{mean}= 47% (CVs for amount,

246 composition and regeneration were 68%, 48% and 26%, respectively) and the morphological
247 traits showed a CV_{mean}= 25% (CVs for body mass and morphometry were 31% and 20%,
248 respectively) across all ten feeding treatments. There were no differences among mean trait
249 variations across resources (Kruskal-Wallis: n= 89, df= 9, χ^2= 2.62, p= 0.98; Fig. 5b), but the
250 variance of trait variation was heteroscedastic among resources (Levene: $F_{9,79}$= 7.60, p<
251 0.0001). Finally, we tested whether the mean total variation of one resource (across all traits)
252 is related to macronutrients in the food (Table 2). We found that trait variation of the resource
253 treatments (see Table 3 for means per resource) were correlated with the C/N-ratio (Spearman
254 rank: ρs= -0.71, p= 0.021, Fig. 6) of the food.

255 ## Discussion

256 Intraspecific reaction norms and variation caused by differing resources, are well-
257 known, yet a mostly neglected topic in evolutionary ecology [2, 29]. Over 100 years ago,
258 Woltereck [29] started to discuss underlying mechanisms, but also noted that it is hardly
259 possible to quantify the range of a species' variability caused by nutritional quality, because
260 even in pure linages of facultative parthenogenetic species, like *Daphnia*, a full control of the
261 genotype is not possible. He also suggested that an obligate asexual species would be a
262 perfect model to study nutritional reaction norms of traits and their plasticity. Parthenogenetic
263 generalists (like some oribatid mites), which are able to survive in a broad range of
264 environmental conditions (i.e. possess a general purpose genotype; [50]), appear to be the
265 models that Woltereck [29] had looked for. Correspondingly, we used our "phenotypically-
266 tractable" model *A. longisetosus* to unravel the relationship of food quality and intraspecific
267 variation of multiple traits (i.e. phenotypic plasticity).

268 *Nutritional effects on traits and variation*

Most studies so far focused on the relationship of nutrients to one or a distinct set of traits – often times related to fitness [e.g., 18, 20, 51, 52]. We think that our multiple traits approach – also including presumable neutral characters – better allows to disentangle patterns and mechanisms of nutrient influence on the mean and variance as well as the inherent plasticity of traits. In our experiment, all traits responded to diet and showed quadratic reaction norms (= optima) of different strength. Physiological theory formally conceptualized this quadratic (or concave) response of fitness relevant performance traits as "threshold elemental ratio" [for element stoichiometry; 25] or as "Bertrand's rule" for essential micronutrients [53] and also macronutrients [18]. Both concepts predict that for low levels of nutrients/elements benefits gained from more increase until a phase of equilibrium is reached. Nutrients/elements beyond this threshold are associated with increasing costs for the regulatory mechanisms resulting in physiological disadvantage higher than the original benefit. Our results for C/N show that a simple threshold elemental ratio applies to multiple traits and, even more important, also to their variability and thus to phenotypic plasticity. This is because the variability of each trait across resources was heteroscedastic, indicating that food quality not only changed the mean [e.g., 54, 55], but also the variation of a trait. These findings could help to propose an general eco-physiological mechanism causing dietary related intraspecific trait variation derived for our model system: the low performance and high plasticity of animals feeding on resources from the "edges" of an elemental or nutritional gradient ingest imbalanced food with stoichiometric shortcomings which causes stress related costs to deal with the deficits as well as surpluses of elements or nutrients [18, 25]. While at low N-content (high C/N ratio) trait performance was limited by a short supply of protein, mites shifted to a C-limited trait performance at high level of N (low C/N). Consequently, high variability of all traits (i.e. high phenotypic plasticity) occurred if either protein (N) or energy (C) limited the formation and performance of traits. More general: if there is a considerable trade-off between consumed surplus and deficit nutrients in one resource, the

295 variation across multiple traits within a phenotype is high. Reciprocally, the intraspecific
296 variation is low at nutritional optima where no essential nutrient is in short supply and a
297 respective genotype can bear its full potential. The concave responses of all traits and their
298 variability to overall food quality (C/N ratio) in our experiments not only indicated that the
299 threshold-elemental-ratio-rule applied for a wide range of traits and their variability, but
300 further suggest costs [20] to maintain a high mean and low variance of a trait. This means that
301 mites feeding on "edge" resources must deal with high costs during allocating resources
302 compared to mite consuming the "optimum" food. Despite these costs, however, variation at
303 the "edges" may still be beneficial, because it enables at least a small number of individuals to
304 survive unfavored conditions. For instance, a recent synthesis by Forman and Wennersten
305 [56] found that variation seems to be more important under stressful circumstances when
306 animals are forced to exist under suboptimal conditions - like stoichiometrically imbalanced
307 food [25] - and may enable the survival of a population [57].

308 *Inherent variability of traits*

309 Generally, there are no multicellular organisms without a certain plasticity, because
310 intra-individual trade-offs as reaction towards environmental conditions like temperature,
311 salinity or resource availability, but also biotic factors like predation will lead to variation of
312 traits [15, 56, 58]. Besides selection, it is still poorly understood why some traits are more
313 prone to variation than others, i.e. bear higher plasticity. Our data indicates that some traits
314 tend to be more variable than other, however this is not necessarily related to a certain "trait
315 type" (in our case "chemistry", "life history" and "morphology"). This different *variability*
316 *potential* allows to derive different hypothesis: a certain trait may react with higher variability
317 to an environmental gradient, because it faces more trade-offs along this gradient than other
318 characters; or the formation/development of a trait may be more "complex" and thus demands
319 a stronger segregation of energy leading to more trade-offs. Consequently, lower trait

variation may indicate less trade-offs (or selection) along a certain gradient. Also, a lower variability may be a signal for an inherently lower plasticity of a trait, because it is less controlled by the phenotypes response or selection. For instance, in our experiments, the body size of the mites changed considerably along the C/N gradient, yet the overall body shape (morphometric measurements) only showed a weak response and low variability. This may indicate, that – besides selection or genetical conservation – the overall body size appeared to be influenced by the phenotypic response to altered nutrients. Yet, the proportions of the body shape isometrically scaled with this phenotypic change, leading to low overall variability.

Conclusions

Overall, we have shown that a threshold elemental ratio, and probably also Bertrand`s rule [18, 25, 53], apply to a wide range of traits and also to dietary caused intraspecific variation of multiple traits (= phenotypic plasticity) in a model system excluding genotypic variation. Thus, there seems not only to be a stoichiometric or nutritional optimum for a traits' mean, but also its variation. Highly imbalanced food yields in higher trait variability, potentially caused by more/stronger trade-offs across resources arising from physiological stress. Additionally, some traits seem to have higher inherent variation than other, irrespective of "trait type". Based on our empirical evidence, we thus would argue that the *mean trait variation* calculated based on all trait variation within a resource could be used as an indicator for the food quality, while the *variation of trait variation* compared across the resources may be an indicator for performance trade-offs [see also 30] while feeding on a respective food. Using a parthenogen excludes genotypic plasticity and creates a phenotypically tractable model species. This is a first step to establish an experimental platform to ultimately disentangle how variation (or the capacity to express plasticity) affects the fitness of individuals [58].

Declarations

Acknowledgments

We thank Andrea Hilpert, Ursula Lebong, Christian Storm, Sonja Elberich, Danny Rothe and Tim Bergmann for experimental assistance as well as Nico Blüthgen for helpful discussion.

Funding

AB was supported by the German Nation Academic Foundation (Studienstiftung des deutschen Volkes). This study was supported by the German Science Foundation (DFG; HE 4593/5-1).

Availability of data and materials

All experimental data can be found in the supplement.

Authors' contributions

MH and AB designed the research; AB, RS and KW performed the experiment, AB performed chemical analyses; AB analyzed the data; AB and MH wrote the paper. All authors discussed and approved the final manuscript.

Ethics approval

There are no legal restrictions on working with mites.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing financial interests.

References

366 1. Mayr E, Provine WB. The evolutionary synthesis: perspectives on the unification of
367 biology. Harvard University Press; 1998.

368 2. Schlichting CD, Pigliucci M. Phenotypic evolution: a reaction norm perspective.
369 Sinauer Associates Incorporated; 1998.

370 3. Whitman DW, Agrawal AA. What is phenotypic plasticity and why is it important.
371 Phenotypic plasticity of insects: Mechanisms and Consequences. 2009:1-63.

372 4. Kelly SA, Panhuis TM, Stoehr AM. Phenotypic plasticity: molecular mechanisms and
373 adaptive significance. Comp Physiol. 2012; 2: 1417-1439.

374 5. Piersma T, Van Gils JA. The flexible phenotype: a body-centred integration of
375 ecology, physiology, and behaviour. Oxford University Press; 2011.

376 6. Fordyce JA. The evolutionary consequences of ecological interactions mediated
377 through phenotypic plasticity. J Exp Biol. 2006; 209:2377-2383.

378 7. Krause S, Le Roux X, Niklaus PA, Van Bodegom PM, Lennon JT, Bertilsson S,
379 Grossart H-P, Philippot L, Bodelier PL. Trait-based approaches for understanding microbial
380 biodiversity and ecosystem functioning. Front Microbiol. 2014; 5:251.

381 8. Violle C, Navas ML, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E. Let the
382 concept of trait be functional! Oikos. 2007; 116:882-892.

383 9. Bjørnstad ON, Hansen TF. Individual variation and population dynamics. Oikos.
384 1994; 69:167-171.

385 10. Grant P, Price T. Population variation in continuously varying traits as an ecological
386 genetics problem. Ame Zool. 1981; 21:795-811.

387 11. Bolnick DI, Amarasekare P, Araújo MS, Bürger R, Levine JM, Novak M, Rudolf VH,
388 Schreiber SJ, Urban MC, Vasseur DA. Why intraspecific trait variation matters in community
389 ecology. Trends Ecol Evol. 2011; 26:183-192.

390 12. Bolnick DI, Svanbäck R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML.
391 The ecology of individuals: incidence and implications of individual specialization. Am Nat.
392 2002; 161:1-28.

393 13. Pachepsky E, Bown JL, Eberst A, Bausenwein U, Millard P, Squire GR, Crawford
394 JW. Consequences of intraspecific variation for the structure and function of ecological
395 communities Part 2: Linking diversity and function. Ecol Model. 2007; 207:277-285.

396 14. Wood CM, McKinney ST, Loftin CS. Intraspecific functional diversity of common
397 species enhances community stability. Ecol Evol. 2017; 7:1553-1560.

398 15. Berg MP, Ellers J. Trait plasticity in species interactions: a driving force of
399 community dynamics. Evo Ecol. 2010; 24:617-629.

400 16. Malerba ME, Heimann K, Connolly SR. Nutrient utilization traits vary systematically
401 with intraspecific cell size plasticity. Func Ecol. 2016; 30:1745-1755.

402 17. Pascacio-Villafán C, Williams T, Birke A, Aluja M. Nutritional and non-nutritional
403 food components modulate phenotypic variation but not physiological trade-offs in an insect.
404 Sci Rep. 2016; 6:srep29413.

405 18. Raubenheimer D, Lee K, Simpson S. Does Bertrand's rule apply to macronutrients?
406 Proc R Soc Lond B. 2005; 272:2429-2434.

407 19. Simpson SJ, Raubenheimer D. The hungry locust. Advances in the Study of Behavior.
408 Volume 29: Elsevier; 2000: 1-44

409 20. Simpson SJ, Raubenheimer D. The nature of nutrition: a unifying framework from
410 animal adaptation to human obesity. Princeton University Press; 2012.

411 21. Wetzel WC, Kharouba HM, Robinson M, Holyoak M, Karban R. Variability in plant
412 nutrients reduces insect herbivore performance. Nature. 2016; 539:425.

413 22. Gibert JP, Allen RL, Hruska RJ, DeLong JP. The ecological consequences of
414 environmentally induced phenotypic changes. Ecol Let. 2017; 20:997-1003.

415 23. Liang D, Silverman J. "You are what you eat": diet modifies cuticular hydrocarbons
416 and nestmate recognition in the Argentine ant, *Linepithema humile*. Naturwissenschaften.
417 2000; 87:412-416.

418 24. Lee KP, Jang T, Ravzanaadii N, Rho MS. Macronutrient balance modulates the
419 temperature-size rule in an ectotherm. Am Nat. 2015; 186:212-222.

420 25. Boersma M, Elser JJ. Too much of a good thing: on stoichiometrically balanced diets
421 and maximal growth. Ecology. 2006; 87:1325-1330.

422 26. Sterner RW, Elser JJ. Ecological stoichiometry: the biology of elements from
423 molecules to the biosphere. Princeton University Press; 2002.

424 27. Heethoff M, Bergmann P, Laumann M, Norton RA. The 20th anniversary of a model
425 mite: A review of current knowledge about *Archegozetes longisetosus* (Acari, Oribatida).
426 Acarologia. 2013; 53:353-368.

427 28. Bergmann P, Laumann M, Norton RA, Heethoff M. Cytological evidence for
428 automictic thelytoky in parthenogenetic oribatid mites (Acari, Oribatida): Synaptonemal
429 complexes confirm meiosis in *Archegozetes longisetosus*. Acarologia. 2018; 58: 342-356.

430 29. Woltereck R. Weitere experimentelle Untersuchungen über Artveränderung, speziell
431 über des Wesen quantitativer Artunterschiede bei *Daphniden*. Ver Deutsche Zool Gesell.
432 1909; 19:110-172.

433 30. Stearns SC. Trade-offs in life-history evolution. Func Ecol. 1989; 3:259-268.

434 31. Scharloo W. Developmental and physiological aspects of reaction norms. BioScience.
435 1989; 39:465-471.

436 32. Norton RA. Evolutionary aspects of oribatid mite life histories and consequences for
437 the origin of the Astigmata. in Mites: Ecological and Evolutionary Analyses of Life-History
438 Patterns. Chapman & Hall edition. Edited by Houck MA. New York: Chapman & Hall; 1994:
439 99-135

440 33. Heethoff M, Raspotnig G. Triggering chemical defense in an oribatid mite using
441 artificial stimuli. Exp Appl Acarol. 2012; 56:287-295.

442 34. Raspotnig G. Chemical alarm and defence in the oribatid mite *Collohmannia gigantea*
443 (Acari: Oribatida). Exp Appl Acarol. 2006; 39:177-194.

444 35. Heethoff M, Koerner L, Norton RA, Raspotnig G. Tasty but protected-first evidence
445 of chemical defense in oribatid mites. J Chem Ecol. 2011; 37:1037-1043.

446 36. Heethoff M, Rall BC. Reducible defence: chemical protection alters the dynamics of
447 predator–prey interactions. Chemoecology. 2015; 25:53-61.

448 37. Peschel K, Norton RA, Scheu S, Maraun M. Do oribatid mites live in enemy-free
449 space? Evidence from feeding experiments with the predatory mite *Pergamasus*
450 *septentrionalis*. Soil Biol Biochem. 2006; 38:2985-2989.

451 38. Jeffries M, Lawton J. Enemy free space and the structure of ecological communities.
452 Biol J Linnean Soc. 1984; 23:269-286.

453 39. Brückner A, Heethoff M. The ontogeny of oil gland chemistry in the oribatid mite
454 *Archegozetes longisetosus* Aoki (Oribatida, Trhypochthoniidae). Int J Acarol. 2017; 43:337-
455 342.

456 40. Heethoff M. Regeneration of complex oil-gland secretions and its importance for
457 chemical defense in an oribatid mite. J Chem Ecol. 2012; 38:1116-1123.

458 41. Dunn OJ. Multiple comparisons using rank sums. Technometrics. 1964; 6:241-252.

459 42. Benjamini Y, Hochberg Y. Controlling the false discovery rate - a practical and
460 powerful approach to multiple testing. J Roy Stat Soc B Met. 1995; 57:289-300.

461 43. Anderson MJ. Permutation tests for univariate or multivariate analysis of variance and
462 regression. Can J Fish Aquat Sci. 2001; 58:626-639.

463 44. Jombart T, Devillard S, Balloux F. Discriminant analysis of principal components: a
464 new method for the analysis of genetically structured populations. BMC Genet. 2010; 11:94.

465 45. Akaike H. A new look at the statistical model identification. IEEE Trans Autom
466 Control. 1974; 19:716-723.

467 46. R Core Team: R: A language and environment for statistical computing. R Foundation
468 for Statistical Computing, Vienna, Austria. 2016.

469 47. Fox J, Weisberg S. An R companion to applied regression. Sage Publications; 2011.

470 48. Pohlert T. The pairwise multiple comparison of mean ranks package (PMCMR). R
471 package. 2014:2004-2006.

472 49. Fox J, Andronic L, Ash M, Boye T, Calza S, Chang A, Grosjean P, Heiberger R,
473 Kerns G, Lancelot R: Rcmdr: R Commander. R package version 1.5-4. 2009.

474 50. Lynch M. Destabilizing hybridization, general-purpose genotypes and geographic
475 parthenogenesis. Q Rev Biol. 1984; 59:257-290.

476 51. Zehnder CB, Hunter MD. More is not necessarily better: the impact of limiting and
477 excessive nutrients on herbivore population growth rates. Ecol Entomol. 2009; 34:535-543.

478 52. Van Amelsvoort P, Usher M. Egg production related to food quality in *Folsomia*
479 *candida* (Collembola: Isotomidae): Effects on life history strategies. Pedobiologia. 1989;
480 33:61-66.

481 53. Bertrand G. On the role of trace substances in agriculture. Eighth Int Congr Appl
482 Chem. 1912; 28:30-40.

483 54. Reger J, Lind MI, Robinson MR, Beckerman AP. Predation drives local adaptation of
484 phenotypic plasticity. Nature Ecol Evol. 2018; 2:100.

485 55. Draghi JA, Whitlock MC. Phenotypic plasticity facilitates mutational variance, genetic
486 variance, and evolvability along the major axis of environmental variation. Evolution. 2012;
487 66:2891-2902.

488 56. Forsman A, Wennersten L. Inter-individual variation promotes ecological success of
489 populations and species: evidence from experimental and comparative studies. Ecography.
490 2016; 39:630-648.

491 57. Price TD, Qvarnström A, Irwin DE. The role of phenotypic plasticity in driving
492 genetic evolution. Proc R Soc Lond B. 2003; 270:1433-1440.

493 58. Forsman A. Rethinking phenotypic plasticity and its consequences for individuals,
494 populations and species. Heredity. 2015; 115:276.

495 59. Blum MS. Chemical Defenses of Arthropods New York: Academic Press, Inc.; 1981.

496 60. Eisner T, Meinwald J. Defensive secretions of arthropods. Science. 1966; 153:1341-
497 1350.

498 61. Pasteels JM, Grégoire J-C, Rowell-Rahier M. The chemical ecology of defense in
499 arthropods. Annu Rev Entomol. 1983; 28:263-289.

500 62. Spencer KC. Chemical mediation of coevolution. San Diego, California, USA:
501 Academic Press, Inc.; 1988.

502 63. Baldwin IT, Dusenbery DB, Eisner T. Squirting and refilling: Dynamics of p-
503 benzoquinone production in defensive glands of *Diploptera punctata*. J Chem Ecol. 1990;
504 16:2823-2834.

505 64. Fescemyer HW, Mumma RO. Regeneration and biosynthesis of dytiscid defensive
506 agents (Coleoptera: Dytiscidae). J Chem Ecol. 1983; 9:1449-1464.

507 65. Stearns SC, Kawecki TJ. Fitness sensitivity and the canalization of life-history traits.
508 Evolution. 1994; 48:1438-1450.

509 66. Brey T, Hain S. Growth, reproduction and production of *Lissarca notorcadensis*
510 (Bivalvia: Philobryidae) in the Weddell Sea, Antarctica. Marine Ecology Progress Series.
511 1992:219-226.

512 67. Thessalou-Legaki M, Kiortsis V. Estimation of the reproductive output of the
513 burrowing shrimp *Callianassa tyrrhena*: a comparison of three different biometrical
514 approaches. Mar Biol. 1997; 127:435-442.

515 68. Brose U, Berlow EL, Martinez ND. Scaling up keystone effects from simple to
516 complex ecological networks. Ecol Let. 2005; 8:1317-1325.

517 69. Jennings S, Mackinson S. Abundance–body mass relationships in size-structured food
518 webs. Ecol Let. 2003; 6:971-974.

519 70. Yodzis P, Innes S. Body size and consumer-resource dynamics. Am Nat. 1992;
520 139:1151-1175.

521 71. Burns J, Di Nardo P, Rodd F. The role of predation in variation in body shape in
522 guppies *Poecilia reticulata*: a comparison of field and common garden phenotypes. J Fish
523 Biol. 2009; 75:1144-1157.

524 72. Sánchez-Hernández J, Vieira-Lanero R, Servia MJ, Cobo F. Feeding habits of four
525 sympatric fish species in the Iberian Peninsula: keys to understanding coexistence using prey
526 traits. Hydrobiologia. 2011; 667:119-132.

527

Figure legends

Fig. 1 Conceptual Fig. depicting the experimental design of this study. Color code corresponds to the ten resources and is used throughout the study. Circles represent sample replicates; sample sizes are also denoted in the figure.

Fig. 2 Secretion amount [ng/µg] of individual mites (a) raised on the ten resources. Conceptual graph (b) showing quadratic response of the secretion amount to the C/N ratio. Fraction of regenerating individuals [%] on each resource (c); the conceptual graphs (d) again show the response of the regeneration probability to the C/N ratio. Black bars represent medians, each filled circle denotes one independent data point. Ordination (discriminant analysis of principal components) of the relative composition of defensive gland exudates [%] of mite individuals reared on the ten resources (e). The center of the stars mark the group mean (centroid), lines indicate the group dispersion (multivariate variance). Percentages in (e) denote the variance explained by each axis. Colors correspond to the Fig. legend in panels (a) and (b). Detailed postdoc comparisons using Dunn's test can be found the supplementary text S2.

Fig. 3 Developmental time [days] of one females offspring, calculated as weight mean (a) raised on the ten resources (note: blood was excluded from the analyses). Conceptual graph (b) showing quadratic response of the secretion amount to the C/N ratio. Survival of one females offspring [%] on each resource (c); the conceptual graphs (d) again show the response of the regeneration probability to the nutritional parameters. Total number of offspring (e) per [N*female^{-1}] and the reproductive output (f) per female [mg*female^{-1}] on the ten resources. The conceptual graphs relate to the response of the total number of offspring (f) or the reproductive output (h) per female to the C/N ratio. Black bars represent medians, each filled circle denotes one independent data point. Colors correspond to the Fig. legend in panels (a)

552 and (b). Detailed postdoc comparisons using Dunn's test can be found the supplementary text
553 S2.

554 **Fig. 4** Body mass [µg] of individual mites (a) raised on the ten resources. Conceptual graph
555 (b) showing quadratic response of the secretion amount to the C/N ratio. Black bars represent
556 medians, each filled circle denotes one independent data point. Ordination (discriminant
557 analysis of principal components) of the elven morphometric characters [% NL] of mite
558 individuals reared on the ten resources (c). The center of the stars mark the group mean
559 (centroid), lines indicate the group dispersion (multivariate variance). Percentages in (c)
560 denote the variance explained by each axis. Colors correspond to the Fig. legend in panels (a)
561 and (b). Detailed postdoc comparisons using Dunn's test can be found the supplementary text
562 S2.

563 **Fig. 5** Variation of the nine selected traits (Table 1) across all ten resources expressed as
564 coefficient of variation [%]. Individual traits are grouped into their respective category
565 (chemistry, life-history or morphology). Circles represent means, lines denote the standard
566 deviation.

567 **Fig. 6** The relationship of the mean total variation [%] of one resource across all traits plotted
568 against the C/N ratio. The black curve denotes a quadratic regression (curvature: 1.514±
569 0.549, t= 2.8, p= 0.028; $\Delta AIC_{\text{to linear regression}}$=5.4), while the grey dotted lines are the 95%
570 confidential intervals of the regression. Circles represent means, colors correspond to the
571 figure legend.

572

573 ## Tables

574 **Table 1** Overview of the nine selected traits of *Archegozetes longisetosus* measured in this study, as well as a definition of each character and
575 general descriptions of these traits in a general ecological context.

	Trait definition (this study)	Trait description	References
Chemistry			
Amount	Amount of defensive secretions of one individuals standardized by its dry weight (ng/µg)	In reservoir based chemical defense the amount is a primary factor to predict how often an animal is able to defend itself against predators and competitors	[59, 60]
Composition	Relative composition (%) of the defensive secretion of one individual	The composition of a defensive chemical blend can determine its effectively against predators, but can also be a consequence of physiological changes/stress of an individual	[59, 61, 62]
Regeneration	Percentage of individuals (%) per group (n=10) which regenerated their defensive secretions over time	The regeneration of defensive secretion is essential to be defended against predators at all, but also to understand the costs of secretory reproduction	[40, 63, 64]
Life-history			
Developmental time	Weighted arithmetic mean developmental time of one females offspring	The first three life-history parameters (developmental time, survival and number of offspring) describe the reproductive fitness	[20, 30, 65]
Survival	Percentage (%) of surviving offspring of one female based on the counted number of laid eggs and newly enclosed adults		
Offspring	Counted number of surviving offspring of one female		
Output	Dry weight of one females entire offspring	The biomass output may also describe the fitness, but also quantifies the ability of a mother to translocate biomass from the resource to her offspring	[66, 67]
Morphology			
Body mass	Dry weight of the initially used females (mothers)	Body mass is an universal predictor of many ecological processes (e.g., metabolism, abundance, or predation)	[68-70]
Morphometry	Elven morphometric characters of the initially used females (for details see supplement)	The shape of an individual plays an important role in basic physiological processes, but also influences predation (by altered handling approaches by predators) and may be used to predict other characters	[22, 71, 72]

576

577 **Table 2** Carbon to nitrogen ratios (mean ± standard deviation; n= 3 replications) of the ten food resources.

	C/N *mean±SD*
blood	3.3±0.017
bone	4.2±0.042
chlorella	5.4±0.023
fungi	11.3±0.005
hemp	5.6±0.048
lupine	7.0±0.033
pollen	13.6±0.252
spirulina	4.3±0.003
wheat	12.4±0.042
yeast	7.5±0.027

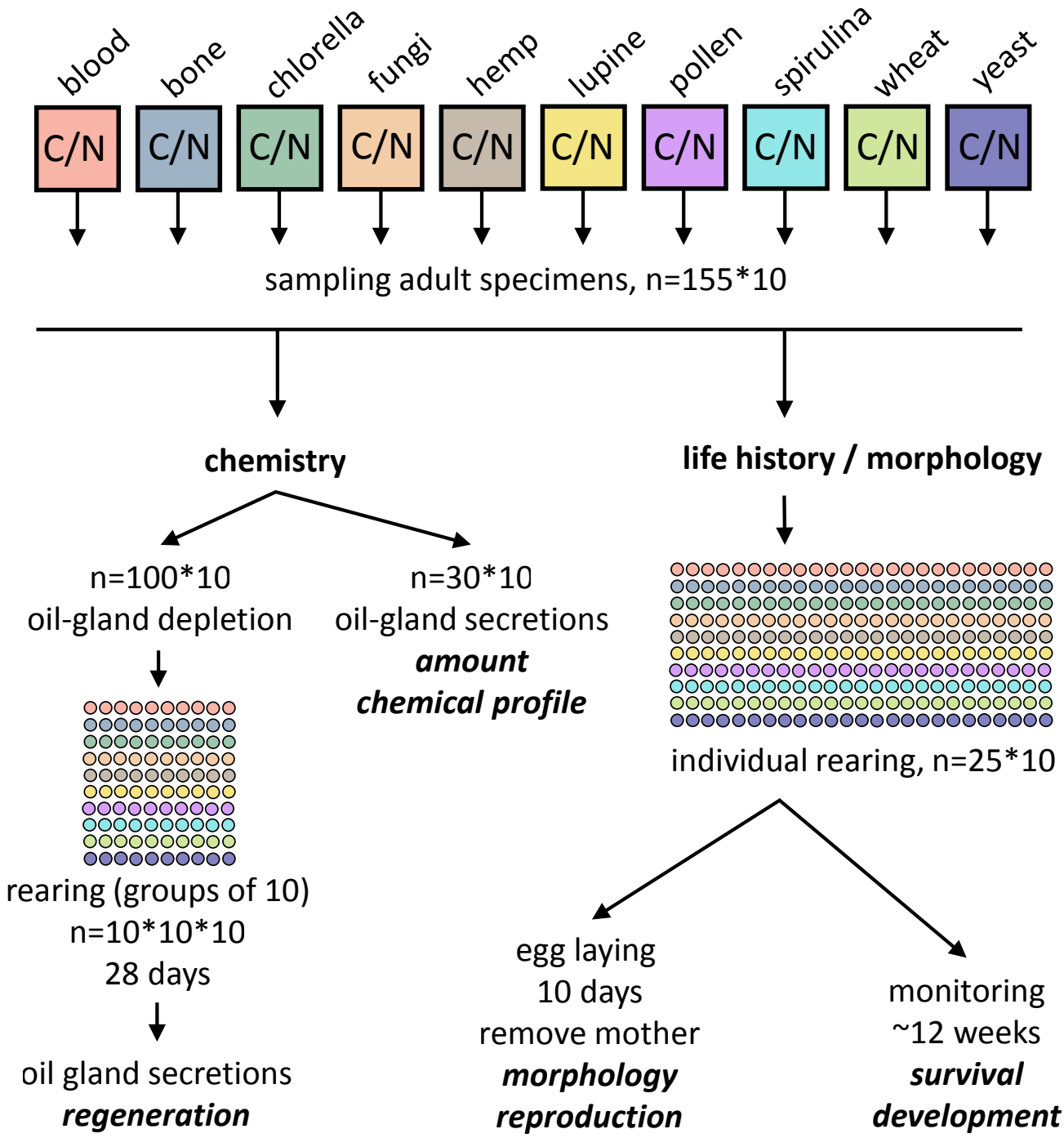
578

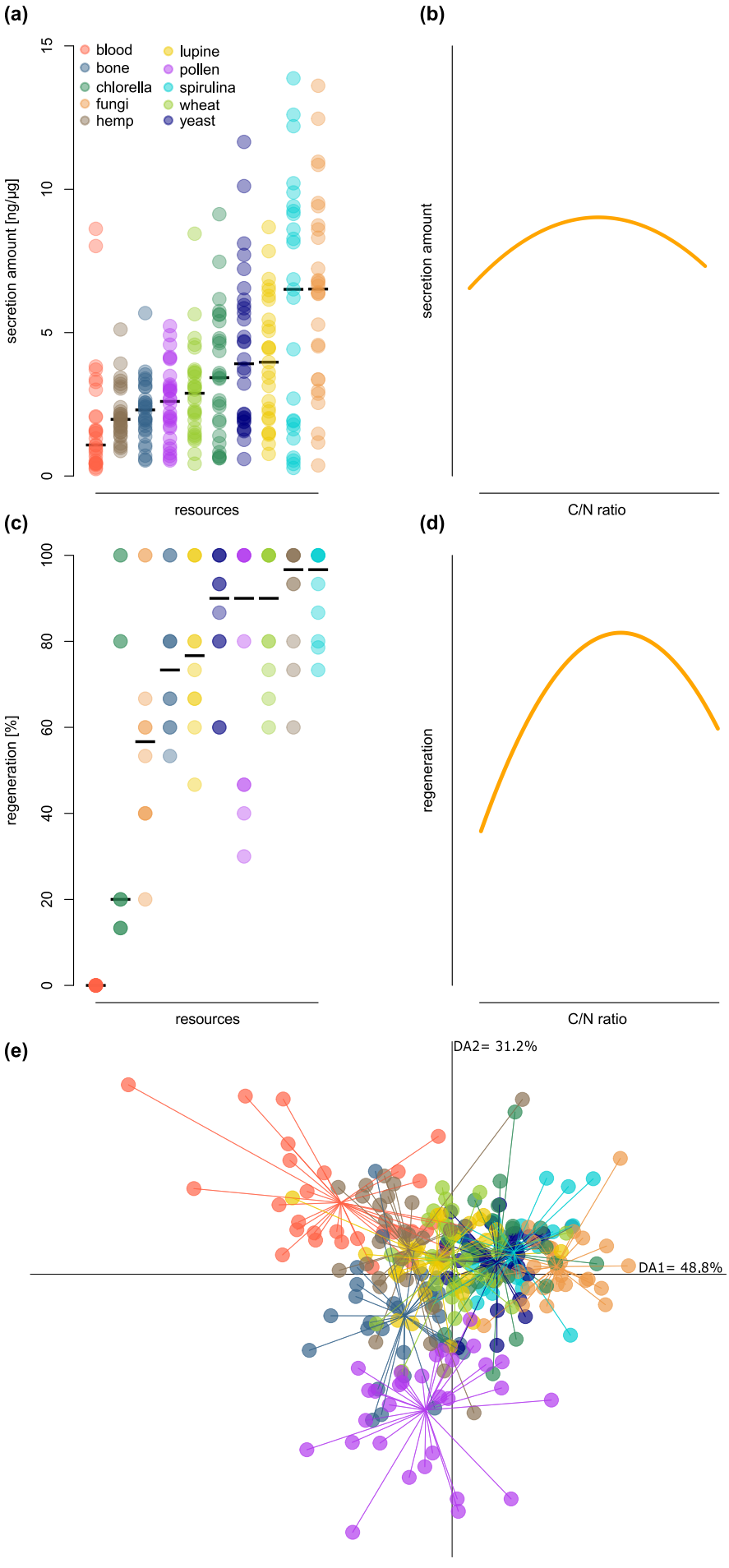
579

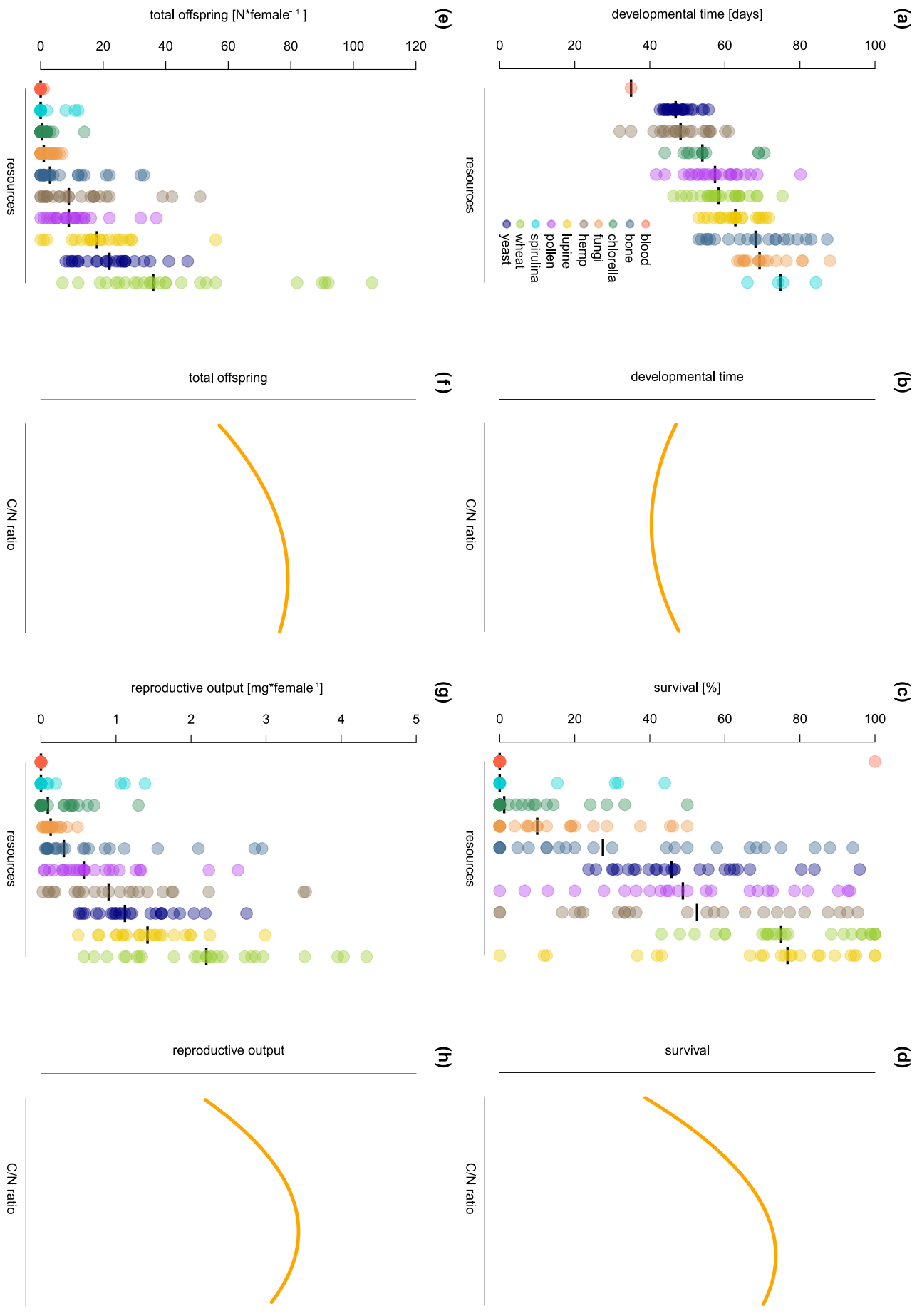
Table 3 Character values of the nine selected traits across the ten resources. Traits in *italics* denoted the categories used for the trait variation analyses. Numbers represent means with standard derivations (SD) and coefficients of variation (CV). Abbreviations: HMBD= 2-hydroxy-6-methylbenzaldehyde, NER= neral, NYF= neryl formate, C13= tridecane, ACA= γ-acaridial (3-hydroxybenzene-1,2-dicarbaldehyde), C15= 7-pentadecene/pentadecane, C17= 6,9-heptadecadiene/8-heptadecene/heptadecane; NL= notogaster length, Nw I= notogaster width I, Nw II= notogaster width II, PL= prodorsum length, Bd= bothridial setae distance, GL= genital plate length, Gw I= genital plate width I, Gw II= genital plate width II, AL= anal plate length, Aw I= anal plate width I, Aw II= anal plate width II. - = not applicable.

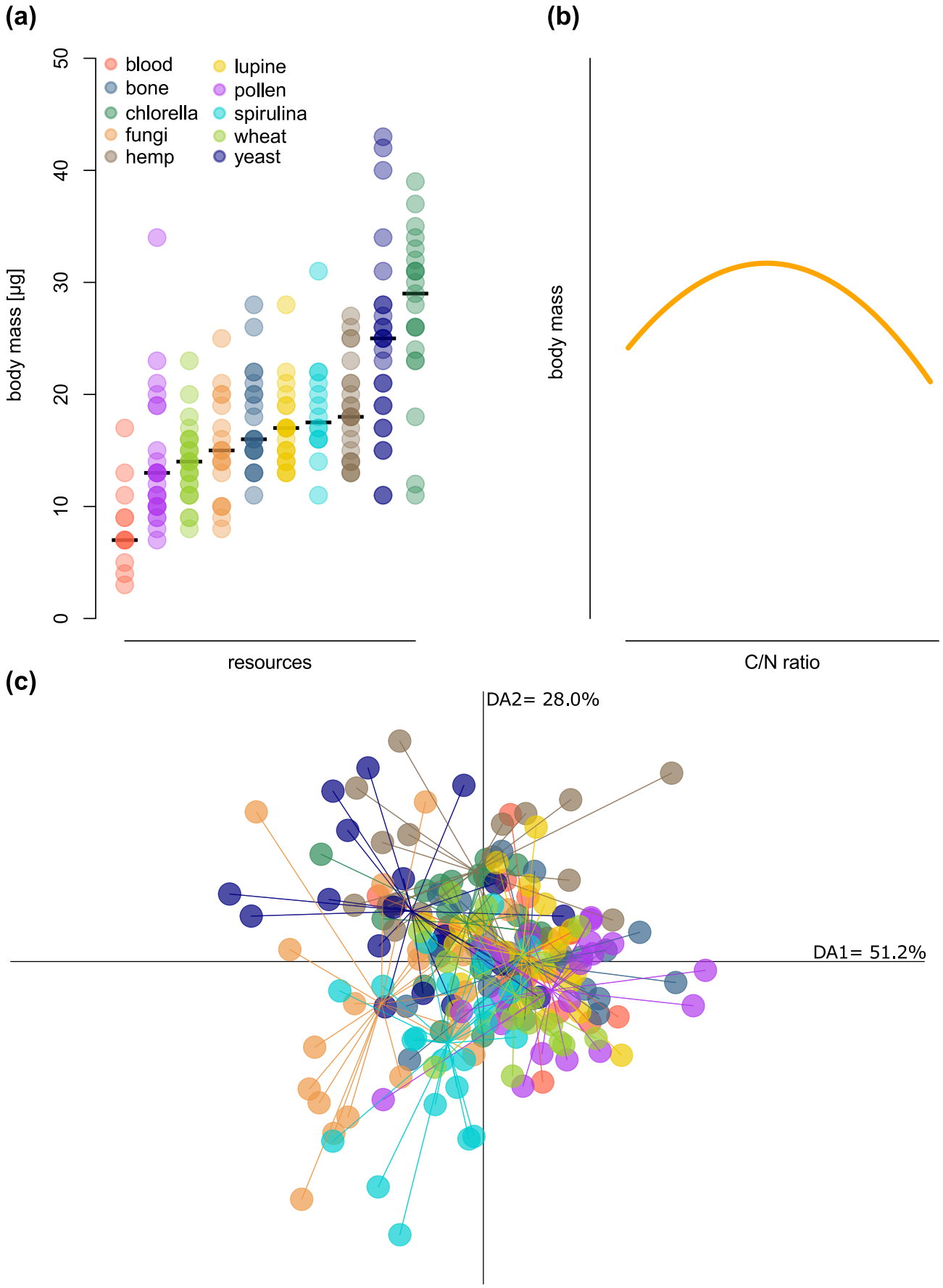
	blood	**bone**	**chlorella**	**fungi**	**hemp**	**lupine**	**pollen**	**spirulina**	**wheat**	**yeast**
	mean±SD (CV)	mean±SD (CV)	mean±SD (CV)	mean±SD (CV)	mean±SD (CV)	mean±SD (CV)	mean±SD (CV)	mean±SD (CV)	mean±SD (CV)	mean±SD (CV)
chemistry										
amount [ng/µg]	2±2(109)	2±1(46)	4±2(64)	6±3(52)	2±1(45)	4±2(54)	2±1(54)	8±10(133)	3±2(57)	4±3(65)
regeneration [%]	0±0 (0)	75±15(21)	46±37(79)	58±25(42)	90±13(15)	77±17(23)	74±28(38)	91±10(11)	86±15(17)	85±15(17)
compositon										
HMBD [%]	4±3(67)	3±1(45)	7±12(166)	12±30(251)	4±2(46)	5±8(170)	6±2(26)	5±5(96)	6±2(36)	6±1(20)
NER [%]	13±8(63)	8±7(87)	19±9(48)	20±21(104)	11±3(29)	12±16(128)	11±4(36)	11±8(70)	16±9(56)	17±4(25)
NYF [%]	36±9(25)	39±11(27)	29±5(18)	34±6(17)	43±5(12)	41±10(25)	35±5(14)	37±10(26)	35±12(35)	30±5(16)
C13 [%]	5±2(29)	12±4(32)	9±5(54)	9±8(85)	8±5(56)	10±5(50)	15±5(34)	10±6(62)	7±2(32)	7±2(23)
ACA [%]	16±4(27)	14±5(36)	21±5(26)	21±6(29)	16±5(31)	15±6(40)	13±5(35)	18±6(33)	18±6(31)	23±4(17)
C15 [%]	12±3(29)	13±3(27)	12±4(37)	12±5(41)	9±9(104)	12±4(33)	9±3(30)	12±5(43)	11±2(21)	10±2(25)
C17 [%]	14±5(35)	12±3(22)	8±5(69)	5±6(127)	9±2(23)	9±4(38)	11±3(27)	7±4(66)	8±2(24)	6±1(23)
life-history										
dev. time [days]	37± - (-)	68±11(15)	56±8(15)	71±7(9)	49±7(15)	63±5(8)	58±8(14)	75±6(9)	59±7(11)	48±3(7)
survival [%]	8±28(30)	37±31(49)	8±13(14)	15±16(18)	50±29(58)	67±29(89)	51±26(54)	11±16(18)	78±18(79)	49±18(36)
offspring [N]	0.1±0.3(332)	8±10(127)	2±3(190)	2±2(102)	14±14(101)	19±12(62)	11±9(81)	3±5(154)	45±26(59)	22±10(45)
output [mg]	0.001±0.002(332)	0.7±0.8(119)	0.3±0.3(125)	0.2±0.1(77)	1.1±0.9(85)	1.5±0.5(38)	0.7±0.6(83)	0.3±0.5(171)	2.2±1.1(46)	1.3±0.6(44)

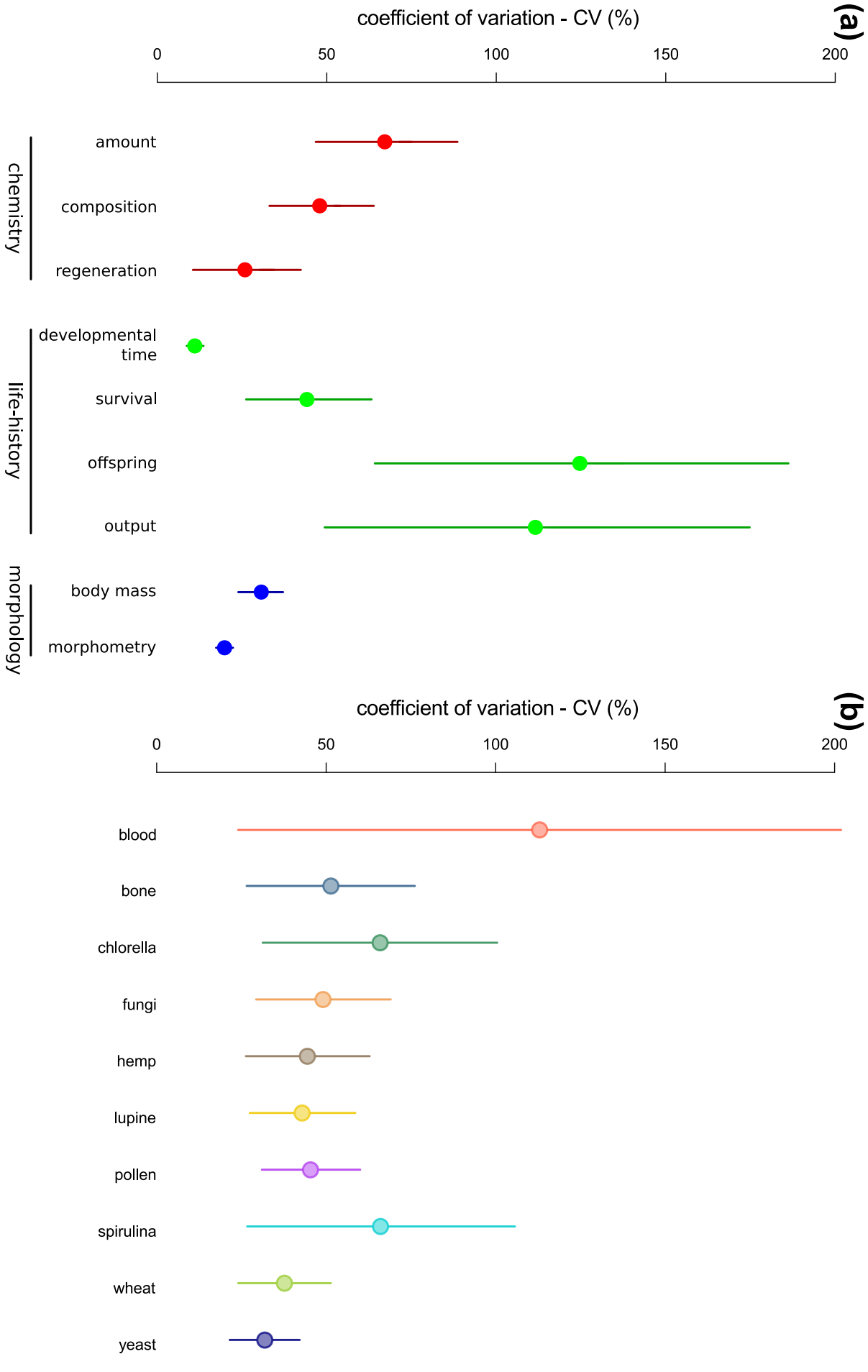
morphology										
body mass [µg]	8±4(46)	17±6(33)	27±9(34)	15±4(30)	19±4(23)	17±3(21)	14±6(42)	19±5(25)	14±3(24)	25±8(35)
morphometry										
Nw I [% NL]	92±16(17)	88±6(7)	92±10(11)	102±15(15)	87±15(17)	83±19(23)	87±9(10)	93±16(17)	91±10(11)	92±14(15)
Nw II [% NL]	63±10(16)	58±5(9)	62±8(13)	61±8(14)	59±6(11)	56±13(23)	58±5(9)	53±8(15)	60±6(11)	60±10(17)
PL [% NL]	32±11(36)	25±8(32)	27±8(32)	35±11(32)	25±8(31)	23±10(44)	26±9(33)	30±12(41)	28±9(31)	27±10(37)
Bd [% NL]	35±8(24)	33±5(15)	31±5(15)	31±7(21)	32±8(25)	30±8(28)	33±5(15)	31±8(26)	33±7(23)	28±6(21)
GL [% NL]	27±4(15)	26±3(10)	26±3(12)	28±5(17)	25±5(18)	24±6(25)	26±3(11)	27±4(16)	27±4(14)	27±4(16)
Gw I [% NL]	20±4(21)	20±3(16)	17±3(16)	22±6(28)	16±3(17)	18±5(27)	20±3(16)	21±5(24)	20±4(19)	19±4(21)
Gw II [% NL]	14±3(20)	13±2(17)	11±2(20)	13±4(31)	11±2(20)	11±3(30)	11±2(21)	13±3(23)	12±1(13)	12±3(24)
AL [% NL]	38±7(19)	36±4(12)	39±7(17)	44±8(18)	40±8(19)	37±9(24)	37±6(17)	40±6(16)	39±6(16)	42±9(21)
Aw I [% NL]	9±2(23)	9±2(24)	8±2(21)	9±2(24)	10±3(28)	8±2(26)	8±2(23)	8±2(20)	8±1(14)	9±2(18)
Aw II [% NL]	14±3(23)	14±3(21)	13±3(19)	15±4(26)	15±3(18)	12±4(28)	12±2(19)	14±3(23)	12±2(16)	15±4(24)
mean CV	114	52	66	50	45	43	46	67	38	32

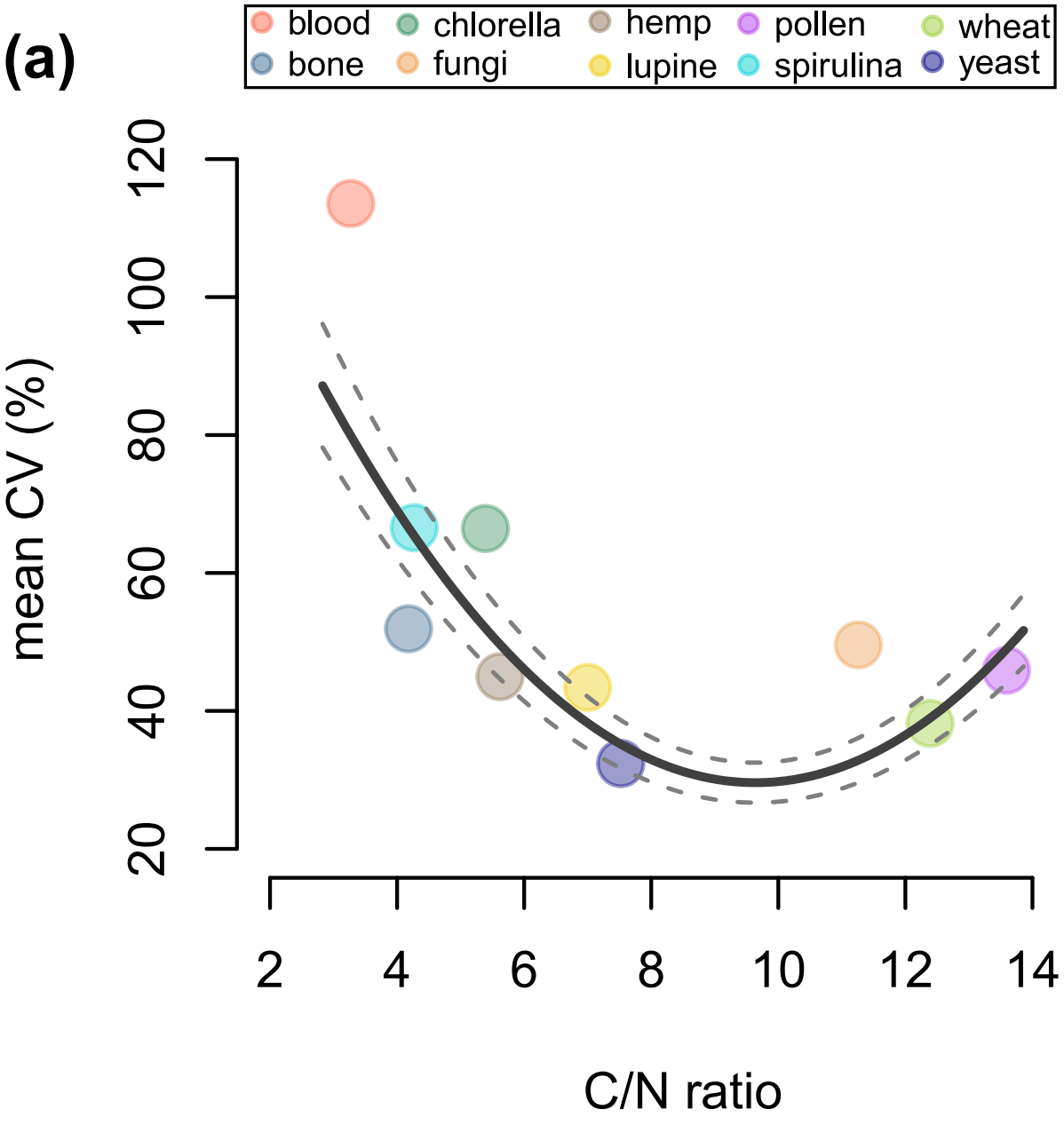

blood
bone
chlorella
fungi
hemp
lupine
pollen
spirulina
wheat
yeast
C/N
sampling adult specimens, n=155*10
chemistry
life history / morphology
n=100*10
oil-gland depletion
n=30*10
oil-gland secretions
amount
chemical profile
individual rearing, n=25*10
rearing (groups of 10)
n=10*10*10
28 days
oil gland secretions
regeneration
egg laying
10 days
remove mother
morphology
reproduction
monitoring
~12 weeks
survival
development


(a)
blood
bone
chlorella
fungi
hemp
lupine
pollen
spirulina
wheat
yeast
secretion amount [ng/µg]
0
5
10
15
resources
(b)
secretion amount
C/N ratio
(c)
regeneration [%]
0
20
40
60
80
100
resources
(d)
regeneration
C/N ratio
(e)
DA2= 31.2%
DA1= 48.8%


(a)
developmental time [days]
0
20
40
60
80
100
resources
blood
bone
chlorella
fungi
hemp
lupine
pollen
spirulina
wheat
yeast
(b)
developmental time
C/N ratio
(c)
survival [%]
0
20
40
60
80
100
resources
(d)
survival
C/N ratio
(e)
total offspring [N*female⁻¹]
0
20
40
60
80
100
120
resources
(f)
total offspring
C/N ratio
(g)
reproductive output [mg*female⁻¹]
0
1
2
3
4
5
resources
(h)
reproductive output
C/N ratio


(a)
blood
bone
chlorella
fungi
hemp
lupine
pollen
spirulina
wheat
yeast
body mass [µg]
0
10
20
30
40
50
resources
(b)
body mass
C/N ratio
(c)
DA2= 28.0%
DA1= 51.2%


coefficient of variation - CV (%)
(a)
0
50
100
150
200
chemistry
amount
composition
regeneration
life-history
developmental time
survival
offspring
output
morphology
body mass
morphometry
coefficient of variation - CV (%)
(b)
0
50
100
150
200
blood
bone
chlorella
fungi
hemp
lupine
pollen
spirulina
wheat
yeast


(a)
blood
bone
chlorella
fungi
hemp
lupine
pollen
spirulina
wheat
yeast
mean CV (%)
20
40
60
80
100
120
2
4
6
8
10
12
14
C/N ratio

10.2 Chemical ecology

10.2.1 Publication 3: **The ontogeny of oil gland chemistry in the oribatid mite *Archegozetes longisetosus* Aoki (Oribatida, Trhypochthoniidae)**

Adrian Brückner and Michael Heethoff

Published in: International Journal of Acarology 43: 337-342

Submitted: 17.03.2017, Accepted: 14.04.2017, Published: 07.06.2017

Authors contributions:

AB and MH designed the research; AB performed chemical analyses; AB analyzed the data; AB and MH wrote the paper. Both authors discussed and approved the final manuscript.

Reprinted by permission from Taylor & Francis Group - an informa business: Taylor & Francis. International Journal of Acarology. The ontogeny of oil gland chemistry in the oribatid mite *Archegozetes longisetosus* Aoki (Oribatida, Trhypochthoniidae). Brückner Adrian, Heethoff Michael. © 2018 Informa UK Limited. Original article archived: https://doi.org/10.1080/01647954.2017.1321042

INTERNATIONAL JOURNAL OF ACAROLOGY, 2017
VOL. 43, NO. 5, 337–342
https://doi.org/10.1080/01647954.2017.1321042

Taylor & Francis
Taylor & Francis Group

Check for updates

The ontogeny of oil gland chemistry in the oribatid mite *Archegozetes longisetosus* Aoki (Oribatida, Trhypochthoniidae)

Adrian Brückner and Michael Heethoff

Ecological Networks, Technische Universität Darmstadt, Darmstadt, Germany

ABSTRACT

The so-called "glandulate Oribatida" (including Astigmata) possess large opisthonotal oil glands, which produce semiochemicals as potent agents for chemical defence against predators (allomones) as well as for intraspecific communication (pheromones). The oil gland reservoirs and their contents remain with shed exuviae, so secretions need to be synthesized *de novo* after each moult. The qualitative composition of chemical profiles may change dramatically between the last juvenile instar (tritonymph) and adult (e.g. *Hermannia convexa* Koch, *Scheloribates azumaensis* Enami, Nakamura & Katsumata, *Oribotritia berlesei* Michael), but reports about quantitative ontogenetic shifts are ambiguous. Here, we analysed the complete ontogenetic sequence (larva, protonymph, deutonymph, tritonymph, and adult) of oil gland secretions in the parthenogenetic oribatid model species *Archegozetes longisetosus* Aoki using gas chromatography–mass spectrometry. We show that absolute and body mass-corrected amounts of oil gland secretions increase during ontogeny and that secretion quantity scales allometrically with body mass (i.e. ontogenetic instar). Furthermore, we found highly significant ontogenetic shifts in the relative quantity of chemical components among instars, but the qualitative composition (=2,6-HMBD, neral, neryl formate, γ-acaridial, tridecane, 7-pentadecene, pentadecane, 6,9-heptadecadiene, 8-heptadecene, and heptadecane) remained stable.

ARTICLE HISTORY
Received 17 March 2017
Accepted 14 April 2017
Published online 3 May 2017

KEYWORDS
Actinotrichida; ecological developmental biology; adult-juvenile chemical polymorphism; chemical ecology; Oribatida

Introduction

Most organisms possess defensive strategies for protection against predators, parasites, and competitors. Chemical defensive secretions are of outmost importance, especially among terrestrial arthropods (Weatherston and Percy 1970; Eisner et al. 2005; Heethoff and Rall 2015). However, the connection between ontogenetic developmental and chemical ecology has rarely been studied. In a pioneer review, Gilbert (2001) noted an urgent need for research at the interface of developmental biology, ecology, and evolution – and this still holds true. While the nymph–adult chemical polymorphism in the hemimetabolous insects, such as true bugs, is relatively well studied (e.g. Aldrich 1988; Leal et al. 1994; Blatt et al. 1998; Prudic et al. 2008), we have little in-depth information on the ontogeny of other arthropod defensive secretions. The few examples include studied mandibulates such as aphids (Mondor et al. 2000), grasshoppers (Blum 1996), julid and polydesmid millipedes (Bodner and Raspotnig 2012; Kuwahara et al. 2015), and stick insects (Dossey et al. 2008).

Among chelicerates, the Sarcoptiformes (acariform mites comprising Oribatida and Astigmata) are characterized by a highly diverse spectrum of natural compounds that are produced by and stored in their opisthonotal oil glands (summarized in Kuwahara 2004 for Astigmata and Raspotnig et al. 2011 for Oribatida). Oribatid mites secrete different classes of chemicals (e.g. hydrocarbons, terpenes, aromatics, esters, alkaloids, and cyanogenic compounds; Brückner et al. 2017) to protect themselves against abiotic environmental factors such as water and soil particles (Brückner et al. 2015) or predators (Heethoff et al. 2011) and some compounds have antimicrobial activity (Raspotnig 2010) as well as a role in intraspecific communication (Shimano et al. 2002; Raspotnig 2006).

Three studies describe ontogenetic changes in the qualitative composition of oribatid oil gland secretions: these relate to *Hermannia convexa* Koch (Raspotnig et al. 2005a), *Scheloribates azumaensis* Enami, Nakamura & Katsumata (Takada et al. 2005), and *Oribotritia berlesei* Michael (Raspotnig et al. 2008). In these species, the nymph–adult chemical dimorphism is characterized by qualitative shifts – reducing, increasing, or replacing a number of compounds in the blend. The few studies that reported quantitative shifts of defensive secretions during ontogeny – i.e. changes only in relative abundances – are ambiguous and lacked proper experimental designs. Shimano et al. (2002) reported a relative shift from nymphs to adults in *Nothrus palustris* Koch, but only one individual from each instar was analysed. Raspotnig et al. (2005b) reported ontogenetically conserved chemical profiles in *Platynothrus peltifer* Koch; they later described a relative ontogenetic shift (Raspotnig et al. 2009) but this was based on only one pooled sample of juveniles. Recently, we studied multivariate patterns of defensive secretions during ontogeny in the parthenogenetic model species *Archegozetes longisetosus* Aoki (Brückner and Heethoff 2017), but absolute amounts of secretions and biological ideas were not reported.

However, the biological consequences of ontogenetic shifts may be significant. On the one hand, secretions need to be synthesized *de novo* after each moult, as the oil gland reservoirs and their contents remain with shed exuviae (Sakata and Norton 2003). Since this loss is frequent – juvenile instars of *A. longisetosus* last for only about 10 days – a low investment in chemical defence of juveniles could be expected. On the other hand, the juveniles of most oribatid mites, including *A. longisetosus*, are not sclerotized, thus chemical defence is potentially of greater importance to them than to adults (Heethoff et al. 2011; Heethoff and Raspotnig 2012; Brückner et al. 2016).

Hence, we studied the ontogenetic sequence of oil gland secretions in *Archegozetes longisetosus*, with the aim of quantifying the investment in chemical defence along the complete ontogenetic sequence (larva, protonymph, deutonymph, tritonymph, and adult), and discuss the biological consequences of relative ontogenetic shifts in defensive compounds in oribatid mites. We show that (i) absolute and relative (compared to body mass) amounts of oil gland secretions increase during

CONTACT Adrian Brückner adrian.brueckner@gmail.com; Michael Heethoff heethoff@bio.tu-darmstadt.de Ecological Networks, Technische Universität Darmstadt, Schnittspahnstraße 3, 64287 Darmstadt, Germany

© 2017 Informa UK Limited, trading as Taylor & Francis Group

ontogeny, supporting the idea of a balanced investment in chemical defence and that (ii) there are highly significant ontogenetic shifts of the relative composition, while the qualitative composition remains quite stable.

Materials and methods

Animals and extraction of oil gland secretions

Specimens of different developmental instars (larvae (LAR), protonymphs (PRO), deutonymphs (DEU), tritonymphs (TRI), adults (ADU)) from the laboratory strain *Archegozetes longisetosus* ran (Heethoff et al. 2007, 2013) were used for chemical analyses. Cultures of *A. longigestosus* ran were kept in constant dark at 28° C and 80–85% relative air humidity. Commercially available powder of dried baker's yeast (*Saccharomyces cerevisiae*; organic quality without food additives; Rapunzel Naturkost, Legau, Germany) was provided *ad libitum* as food on filter paper, three times a week.

For the extraction of oil gland secretions, specimens were haphazardly selected and removed from their culture plates. Clean, actively moving mites were extracted in groups of five (with 20 replicates each) for ADU, TRI, and DEU and 20–25 individuals (with five replicates each) for PRO and LAR by immersing in 50 and 12.5 µl hexane (GC grade, 98% purity purchased from Merck, Darmstadt, Germany) for 3 min, respectively. The extraction solvent also contained tetradecane (1 ng/µl; ≥99.8%, analytical standard, purchased from Sigma-Aldrich, Munich, Germany) as an internal standard. Crude extracts were used for gas chromatography–mass spectrometry (GC/MS) analyses and mite specimens were stored at −20°C for further measurements.

Chemical analysis with GC-MS

Oil gland secretion samples were analysed with a QP 2010 ultra GC-MS (Shimadzu, Duisburg, Germany). The gas chromatograph was equipped with a ZB-5MS fused silica capillary column (30 m × 0.25 mm ID, df = 0.25 µm) from Phenomenex (Aschaffenburg, Germany); 4 µl (for ADU, TRI, DEU and PRO) or 5 µl (for LAR) sample aliquots were analysed as described by Brückner and Heethoff (2016). Tetradecane with a concentration of β = 1 ng/µl was used for quantification of oil gland secretion (for example chromatogram, see Figure 1). In the range of relevant concentrations, the responses of all compounds relative to tetradecane were determined by a dilution series of a pooled crude extract and turned out to be close to 1, allowing an approximation of absolute quantities of oil gland secretions in ng. Equation (1) was used to quantify the individual amounts of oil gland secretions of single specimens (with β as mass concentration of the internal standard, V_s as sample volume, and n as number of extracted specimens):

$$\text{amount(in ng)} = \frac{\beta(\text{internal standard}) \times \sum \text{area(oil gland compounds)} \times V_s}{\text{area(internal standard)} \times n} \quad (1)$$

The chemical composition of the oil gland secretion of *A. longisetosus* has been described earlier (Sakata and Norton 2003; Raspotnig and Föttinger 2008; Heethoff and Raspotnig 2011). The oil gland secretions comprise the following compounds (Figure 1(h)): 2,6-HMBD (=2-hydroxy-6-methyl-benzaldehyde), neral (and sometimes geranial), neryl formate, γ-acaridial (=3-hydroxybenzene-1,2-dicarbaldehyde), tridecane, pentadecene, pentadecane, heptadecadiene, heptadecene and heptadecane (Heethoff and Raspotnig 2011). However, the double-bond positions in the alkenes have not been determined. We used dimethyl-disulfide (DMDS) adduct derivatization as described by Dunkelblum et al. (1985) to elucidate the positions in pentadecene, heptadecene, and heptadecadiene. Oil gland secretions of *Oribotritia berlesei* (Raspotnig et al. 2008) were used as a natural source of 6,9-heptadecadiene and 8-heptadecene for chromatographic comparison. Specimens of *O. berlesei* were collected from litter samples near Ferlach, Carinthia, Austria (46°31′31.4″N 14°19′12.1″E) in May 2015 (leg. A. Brückner). Oil gland secretions were extracted and measured as described above.

Body mass measurement and data processing

Extracted mite specimens were dried at 60°C. Body mass (dry weight) of each instar was determined with a microbalance (Mettler Toledo, XS3DU, 0.1 µg readability and 1 µg repeatability). Groups of 5, 10, 15, 20, and 25 specimens of each developmental instar were measured and the mean dry weight of each instar was calculated. Peak areas of the compounds and of the internal standard expressed as total ion current (TIC) were measured using the same integration criteria for all developmental instars. Absolute amounts (total secretion in ng) were calculated as given in Equation (1); corrected amounts (total secretion per animal dry weight in ng/µg) were calculated by relating the absolute amounts to measured body masses; and relative amounts (proportions) of single compounds were calculated directly from the TIC data. For statistical analyses, C15:1 and C15 as well as C17:2, C17:1, and C17 were combined to a pooled C15 and C17 fraction, respectively.

Statistical analysis

For statistical comparison of total and body-mass-corrected secretions among the developmental instars as well as the comparison of the main compound proportions among the instars, Kruskal–Wallis tests (Kruskal and Wallis 1952) with affiliated pairwise Mann–Whitney U tests (Mann and Whitney 1947) and false-discovery rate (Benjamini and Hochberg 1995) were calculated. Furthermore, we modelled the relationship of defensive secretion amount [ng] per dry weight [µg] as follows: the secretion data were not normally distributed, but cannot be negative, hence this indicated a log-normal distribution of the secretion amount (Shapiro–Wilk test on $\log_{10}$-transformed data: $p = 0.12$). Thus, we used Equation (2), where a is the scaling exponent of the power law and k is a coefficient.

$$\log_{10}(\text{secretion amount[ng]}) = \log_{10}(k \times \text{dry weight[µg]}^a) \quad (2)$$

We modelled and analysed the data with the generic non-linear least squares function using Equation (2) while assuming zero secretions for the dry weight zero. All statistics were performed with R version 3.2.3 codenamed "Wooden Christmas Tree" (R Core Development Team 2015).

Results

General description of the ontogeny of the chemical profiles

Chromatographic profiles of the five developmental instars of *A. longisetosus* showed identical chemical components but differences in their relative amounts (Figure 1(a–g)). While neryl formate followed a strict trend from high to low concentrations along the course of development (Kruskal–Wallis test: $H_{4,63} = 45.40$, $p < 0.001$; Figure 1(c)), γ-acaridial showed the opposite pattern with increasing concentrations in older instars (Kruskal–Wallis test: $H_{4,63} = 31.03$, $p < 0.001$; Figure 1(e)). Hence, while neryl formate was the main compound in all juvenile instars, γ-acaridial was the main compound in ADU. The concentration of neral (Kruskal–Wallis test: $H_{4,63} = 34.45$, $p < 0.001$; Figure 1(b)) and 2,6-HMBD (Kruskal–Wallis test: $H_{4,63} = 58.11$, $p < 0.001$; Figure 1(a)) also significantly differed among the instars, but there was no trend along the developmental course. The hydrocarbon fractions (Figure 1(d,f,g)) also differed among instars (Kruskal–Wallis test: C13: $H_{4,63} = 40.48$, $p < 0.001$, Figure 1(d); C15: $H_{4,63} = 54.11$, $p < 0.001$, Figure 1(f); C17: $H_{4,63} = 55.37$, $p < 0.001$, Figure 1(g)). While the C17 fraction was dominant in juveniles, C13 and C15 were lower

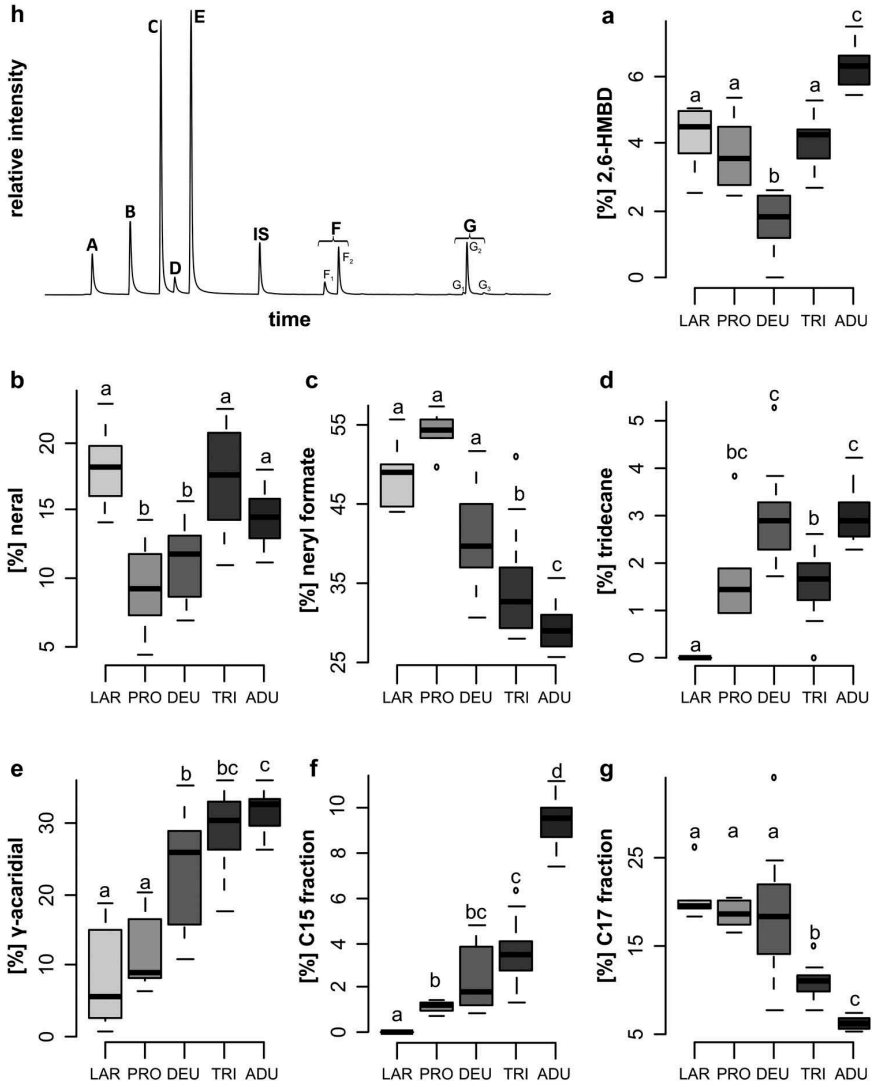


Figure 1. Relative proportions (a–g) of different oil gland compounds during the five developmental instars; the boxplots are presented in order of the retention times. Letters indicate homogenous groups (pairwise Mann–Whitney U tests with FDR-adjusted *p*-values). An exemplary chromatographic profile (h) from a hexane extract of an adult individual of *Archegozetes longesitosus* kept on yeast. Chromatographic profile from a hexane extract of an adult individual of *Archegozetes longesitosus* kept on yeast. In order of retention times: A – 2,6-HMBD, B – neral, C – neryl formate, D – tridecane, E – γ-acaridial, IS – internal standard (tetradecane 1 ng/µl), F_1 – 7-pentadecene, F_2 – pentadecane, G_1 – 6,9-heptadecadiene, G_2 – 8-heptadecene, G_3 – heptadecane.

in earlier developmental instars, becoming successively more dominant towards ADU.

Amount of oil gland secretion during ontogeny

As expected, ADU produced the highest absolute amounts of secretion, followed by TRI (about one-tenth as much), DEU (half as much as TRI), PRO, and LAR (10–15 times less secretion than DEU; Figure 2(a); Kruskal–Wallis test: $H_{4,63} = 58.84$, $p < 0.001$; Table 1). The mean body masses (dry weight) were 65 µg for ADU, 15 µg for TRI, 9 µg for DEU, 3 µg for PRO, and 2 µg for LAR. Corrected secretion amounts (measured total secretion divided by body mass) also differed among developmental instars (Figure 2(b); Kruskal–Wallis test: $H_{4,63} = 52.88$, $p < 0.001$) with LAR and PRO having the lowest, DEU and TRI intermediate, and ADU the highest values (Table 1). Furthermore, the amount of defensive secretion [ng] scaled allometrically with body mass [µg] (Figure 2(c)) based on the power law (3)

$$\text{defense secretion}[\text{ng}] = k \times \text{dry weight}[\mu\text{g}]^{\alpha} \quad (3)$$

for $k = 0.22$ ($t = 7.8$, $p < 0.001$) and $\alpha = 1.66$ ($t = 37.6$, $p < 0.001$).

Double-bond positions

The DMDS adduct of C15:1 (peak F_1, Figure 1(h)) showed a molecular ion at m/z = 304 and diagnostic ions at m/z = 145 and m/z = 159 and was thus identified as 7-pentadecene. Due to the low amount of C17:2 (peak G_1, Figure 1(h)) no DMDS adducts were detected after derivatization, but comparison of retention time and mass spectrometric fragmentation patterns with those of *O. berlesei* (Raspotnig et al. 2008) indicated the same C17:2 (6,9-heptadecadiene) in the secretions of both species. Peak G_2 (C17:1, Figure 1(h)) had a DMDS adduct at m/z = 332 and diagnostic ions at m/z = 159 and m/z = 173, corresponding to 8-heptadecene. In addition, retention time and mass spectrum were identical to 8-hepatdecene from *O. berlesei* (Raspotnig et al. 2008).

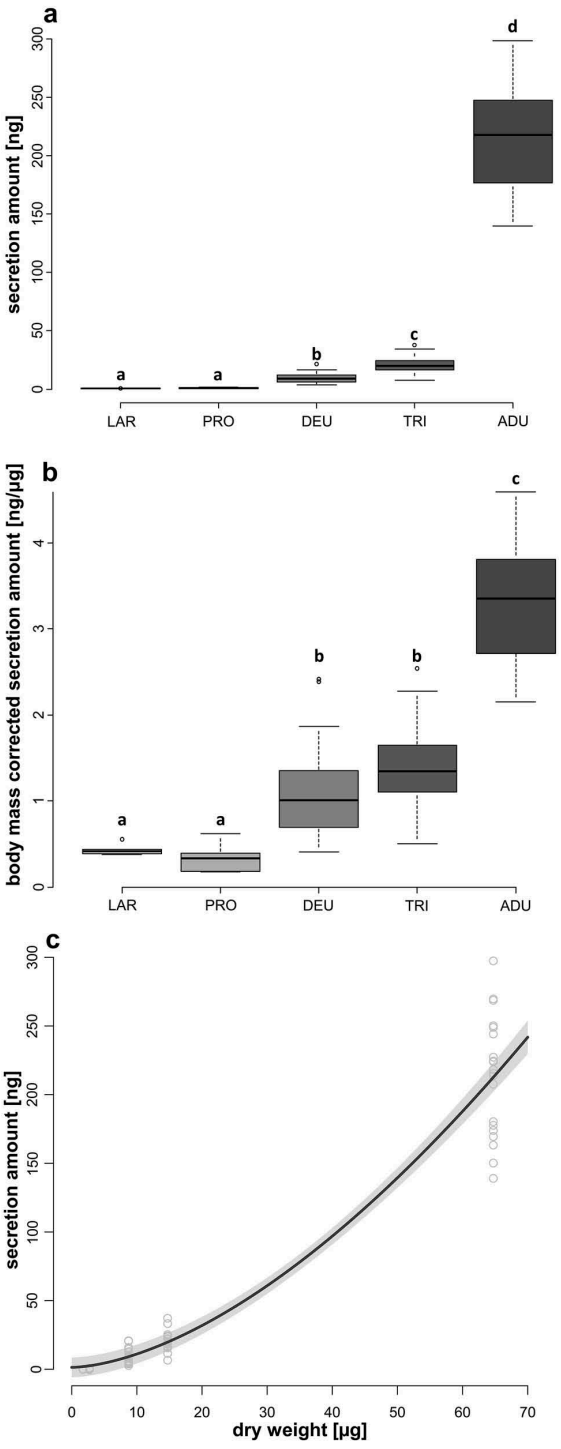


Figure 2. Absolute amount (a) and corrected amount (b) of total secretion in ng and ng/µg of the different developmental instars of *Archegozetes longesitosus*, respectively. Letters indicate homogenous groups (pairwise Mann–Whitney U tests with FDR-adjusted *p*-values). Allometric power law (c) based on the amount of defensive secretion [ng] scaled with the body mass [µg].

Discussion

Our results show that the qualitative composition of the defensive secretion remains almost stable during the development of *A. longisetosus*, but their relative amounts change significantly. In the first examination of oil gland chemistry in this species, Sakata and Norton (2003) found no evidence for a major ontogenetic shift in composition, but they investigated a comparatively small sample size without quantification or statistical analyses. Since the qualitative composition is indeed identical among all instars, relative differences become discernible only through greater replication, proper quantification, and statistical analysis. The relative proportion of neryl formate was higher in all juvenile instars than in ADU, especially in LAR, PRO, and DEU. This also was reported by Sakata and Norton (2003) for LAR of *A. longisetosus* and by Raspotnig et al. (2009) for an undetermined juvenile instar of *P. peltifer*, hence this may be a consistent pattern. This early-instar occurrence of neryl formate supports the hypothesis by Heethoff (2012) that neryl formate may be a *de novo* synthesized precursor of neral. The idea was supported by Morita et al. (2004), who found that neryl formate appeared during the biosynthesis of oil gland secretions in the astigmatid mite *Carpoglyphus lactis* L, but was absent in the final chemical blend, which only contained neral. While γ-acaridial showed a clear, but opposite developmental pattern, there was no trend for neral or 2,6-HMBD even though they differed significantly among instars. The various hydrocarbons were present in all ontogenetic instars, except tridecane and the C15 fraction was not detectable in LAR. While the relative amount of the C17 fraction decreased during the development, tridecane and the C15 fraction increased. This suggests the C17 hydrocarbons to be the main solvent of the oil gland secretion, rather than the C15 fraction as suggested by Heethoff (2012), but probably, all hydrocarbons contribute as solvents.

The erratic patterns (neither increase nor decrease) of neral and 2,6-HMBD may be explained by different regeneration dynamics (and therefore altered enzyme activity in juvenile instars and ADU) of the different compounds (Heethoff 2012). In ADU, oil gland reservoirs only need to be partially refilled after discharge, e.g. the attack of a predator (Heethoff 2012; Heethoff and Rall 2015). In contrast, juvenile mites lose the gland and its contents at each moult and must restore the secretions quickly to regain their chemical defence against predators (Heethoff and Raspotnig 2012). This may explain why the variability of oil gland profiles is lower in ADU and higher in juvenile instars; ADU can regenerate their secretions over a longer period of time, while juvenile instars need a quicker and therefore, perhaps more unbalanced biosynthesis.

All previous studies on the gland chemistry of *A. longisetosus* left the double-bond position unelucidated (Sakata and Norton 2003; Raspotnig and Föttinger 2008; Heethoff and Raspotnig 2011). We determined these substances as 7-pentadecene, 6,9-heptadecadiene, and 8-heptadecene which are widely distributed among Oribatida (Raspotnig 2010) and Astigmata (Kuwahara 2004) and considered to be a symplesiomorphic character in oil gland secretions of both groups (Raspotnig 2010).

As expected, earlier instars produced less total defensive secretion than later instars and the increased amount of secretion can simply be explained by an increase of body mass. While total amounts in LAR and PRO are similar, the amount per body mass allometrically increased towards the later instars (power law: secretion = 0.22 × body mass$^{1.66}$; see results and Figure 2(c)). This may have two reasons: (i) juvenile mites synthesize less defensive secretion only because they have to resynthesize their chemical arsenal after each moult (Sakata and Norton 2003), also juvenile instars only last about 10–12 days (Heethoff et al. 2007), hence larvae and

Table 1. Amount of oil gland secretion during ontogeny. Numbers are means ± standard derivation.

	Secretion amount [ng]	Body-mass-corrected amount [ng/µg]
LAR	0.68 ± 0.13	0.34 ± 0.07
PRO	1.01 ± 0.49	0.34 ± 0.17
DEU	10.24 ± 5.35	1.14 ± 0.59
TRI	20.61 ± 7.60	1.37 ± 0.51
ADU	214.11 ± 43.07	3.29 ± 0.66

nymphs are not able to synthesize higher amounts of defensive secretion during this period, even if the glands grow isometrically with body mass, or (ii) juveniles need their metabolic energy for growth and only invest in a an essential chemical protection.

Acknowledgments

We would like to thank Lukas Kauling for experimental assistance, Sebastian Schmelzle, Roy A. Norton, and another anonymous reviewer for helpful suggestions.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Adrian Brückner was supported by a PhD scholarship from the German National Academic Foundation (Studienstiftung des deutschen Volkes). This study was funded by the German Research Foundation (DFG) [HE 4593/5-1].

References

Aldrich JR. 1988. Chemical ecology of the Heteroptera. Annual Review of Entomology 33:211–238.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society, Series B 57:289–300.

Blatt SE, Borden JH, Pierce HD, Gries R, Gries G. 1998. Alarm pheromone system of the western conifer seed bug, *Leptoglossus occidentalis*. Journal of Chemical Ecology 24:1013–1031.

Blum MS. 1996. Semiochemical parsimony in the Arthropoda. Annual Review of Entomology 41:353–374.

Bodner M, Raspotnig G. 2012. Millipedes that smell like bugs: (E)-alkenals in the defensive secretion of the julid diplopod *Allajulus dicentrus*. Journal of Chemical Ecology 38:547–556.

Brückner A, Heethoff M. 2016. Scent of a mite: origin and chemical characterization of the lemon-like flavor of mite-ripened cheeses. Experimental and Applied Acarology 69:249–261.

Brückner A, Heethoff M. 2017. A chemo-ecologists' practical guide to compositional data analysis. Chemoecology 27:33–46.

Brückner A, Raspotnig G, Wehner K, Meusinger R, Norton RA, Heethoff M. 2017. Storage and release of hydrogen cyanide in a chelicerate (*Oribatula tibialis*). Proceedings of the National Academy of Sciences of the United States of America 114:3469–3472.

Brückner A, Stabentheiner E, Leis H-J, Raspotnig G. 2015. Chemical basis of unwettability in Liacaridae (Acari, Oribatida): specific variations of a cuticular acid/ester-based system. Experimental and Applied Acarology 66:313–335.

Brückner A, Wehner K, Neis MHeethoff M. 2016. Attack and defense in a gamasid-oribatid mite predator-prey experiment – sclerotization outperforms chemical repellency. Acarologia 56:451-461.

Dossey AT, Walse SS, Edison AS. 2008. Developmental and geographical variation in the chemical defense of the walkingstick insect *Anisomorpha buprestoides*. Journal of Chemical Ecology 34:584–590.

Dunkelblum E, Tan SH, Silk PJ. 1985. Double-bond location in monounsaturated fatty acids by dimethyl disulfide derivatization and mass spectrometry: application to analysis of fatty acids in pheromone glands of four Lepidoptera. Journal of Chemical Ecology 11:265–277.

Eisner T, Eisner M, Siegler M. 2005. Defenses of insects, spiders, scorpions, and other many-legged creatures. Cambridge (MA): The Belknap Press of Harvard University Press.

Gilbert SF. 2001. Ecological developmental biology: developmental biology meets the real world. Developmental Biology 233:1–12.

Heethoff M. 2012. Regeneration of complex oil-gland secretions and its importance for chemical defense in an oribatid mite. Journal of Chemical Ecology 38:1116–1123.

Heethoff M, Bergmann P, Laumann M, Norton RA. 2013. The 20th anniversary of a model mite: a review of current knowledge about *Archegozetes longisetosus* (Acari, Oribatida). Acarologia 53:353–368.

Heethoff M, Koerner L, Norton RA, Raspotnig G. 2011. Tasty but protected – first evidence of chemical defense in oribatid mites. Journal of Chemical Ecology 37:1037–1043.

Heethoff M, Laumann M, Bergmann P. 2007. Adding to the reproductive biology of the parthenogenetic oribatid mite, *Archegozetes longisetosus* (Acari, Oribatida, Trhypochthoniidae). Turkish Journal of Zoology 31:151–159.

Heethoff M, Rall BC. 2015. Reducible defence: chemical protection alters the dynamics of predator–prey interactions. Chemoecology 25:53–61.

Heethoff M, Raspotnig G. 2011. Is 7-hydroxyphthalide a natural compound of oil gland secretions? – evidence from *Archegozetes longisetosus* (Acari, Oribatida). Acarologia 51:229–236.

Heethoff M, Raspotnig G. 2012. Expanding the 'enemy-free space' for oribatid mites: evidence for chemical defense of juvenile *Archegozetes longisetosus* against the rove beetle *Stenus juno*. Experimental and Applied Acarology 56:93–97.

Kruskal WH, Wallis WA. 1952. Use of ranks in one-criterion variance analysis. Journal of the American Statistical Association 47:583–621.

Kuwahara Y. 2004. Chemical ecology of astigmatid mites. In: Cardé RT, Millar JG, editors. Advances in insect chemical ecology. Cambridge (UK): Cambridge University Press. p. 76–109.

Kuwahara Y, Ichiki Y, Morita M, Tanabe T, Asano Y. 2015. Chemical polymorphism in defense secretions during ontogenetic development of the millipede *Niponia nodulosa*. Journal of Chemical Ecology 41:15–21.

Leal WS, Panizzi AR, Niva CC. 1994. Alarm pheromone system of leaf-footed bug *Leptoglossus zonatus* (Heteroptera: Coreidae). Journal of Chemical Ecology 20:1209–1216.

Mann H, Whitney D. 1947. On a test of whether one of two random variables is stochastically larger than the other. The Annals of Mathematical Statistics 18:50–60.

Mondor EB, Baird DS, Slessor KN, Roitberg BD. 2000. Ontogeny of alarm pheromone secretion in pea aphid, *Acyrthosiphon pisum*. Journal of Chemical Ecology 26:2875–2882.

Morita A, Mori N, Nishida R, Hirai N, Kuwahara Y. 2004. Neral (the alarm pheromone) biosynthesis via the mevalonate pathway, evidence by D-glucose-1-^{13}C feeding in *Carpoglyphus lactis* and ^{13}C incorporation into other opisthonotal gland exudates. Journal of Pesticide Science 29:27–32.

Prudic KL, Noge K, Becerra JX. 2008. Adults and nymphs do not smell the same: the different defensive compounds of the giant mesquite bug (*Thasus neocalifornicus*: Coreidae). Journal of Chemical Ecology 34:734–741.

R Core Team. 2015. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. Available from: http://www.R-project.org/

Raspotnig G. 2006. Chemical alarm and defence in the oribatid mite *Collohmannia gigantea* (Acari: Oribatida). Experimental and Applied Acarology 39:177–194.

Raspotnig G, Föttinger P. 2008. Analysis of individual oil gland secretion profiles in oribatid mites (Acari: Oribatida). International Journal of Acarology 34:409–417.

Raspotnig G, Kaiser R, Stabentheiner E, Leis H-J. 2008. Chrysomelidial in the opisthonotal glands of the oribatid mite, *Oribotritia berlesei*. Journal of Chemical Ecology 34:1081–1088.

Raspotnig G, Krisper G, Schuster R. 2005a. Ontogenetic changes in the chemistry and morphology of oil glands in *Hermannia*

convexa (Acari: Oribatida). Experimental and Applied Acarology 35:47–58.

Raspotnig G, Krisper G, Schuster R, Fauler G, Leis H-J. 2005b. Volatile exudates from the oribatid mite, *Platynothrus peltifer*. Journal of Chemical Ecology 31:419–430.

Raspotnig G, Norton RA, Heethoff M. 2011. Oribatid mites and skin alkaloids in poison frogs. Biology Letters 7:555–556.

Raspotnig G. 2010. Oil gland secretions in Oribatida (Acari). In: Sabelis MW, Bruin J, editors. Trends in acarology. Dordrecht (Netherlands): Springer. p. 235–239.

Raspotnig G, Stabentheiner E, Föttinger P, Schaider M, Krisper G, Rechberger G, Leis HJ. 2009. Opisthonotal glands in the Camisiidae (Acari, Oribatida): evidence for a regressive evolutionary trend. Journal of Zoological Systematics and Evolutionary Research 47:77–87.

Sakata T, Norton RA. 2003. Opisthonotal gland chemistry of a middle-derivative oribatid mite, *Archegozetes longisetosus* (Acari: Trhypochthoniidae). International Journal of Acarology 29:345–350.

Shimano S, Sakata T, Mizutani Y, Kuwahara Y, Aoki J. 2002. Geranial: the alarm pheromone in the nymphal stage of the oribatid mite, *Nothrus palustris*. Journal of Chemical Ecology 28:1831–1837.

Takada W, Sakata T, Shimano S, Enami Y, Mori N, Nishida R, Kuwahara Y. 2005. Scheloribatid mites as the source of pumiliotoxins in dendrobatid frogs. Journal of Chemical Ecology 31:2403–2415.

Weatherston J, Percy JE. 1970. Studies of physiologically active arthropod secretions. IV. Topography of the sex pheromone producing gland of the eastern spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Canadian Journal of Zoology 48:569–571.

10.2.2 Publication 4: **Nutritional effects on chemical defense alter predator-prey dynamics**

Adrian Brückner and Michael Heethoff

Published in: Chemoecology 28: 1-9

Submitted: 05.12.2017, Accepted: 15.02.2017, Published: 07.03.2018

Authors contributions:
AB and MH designed the research; AB performed chemical analyses; AB analyzed and modeled the data; AB and MH wrote the paper. Both authors discussed and approved the final manuscript.

Reprinted by permission from Springer Nature: Springer International Publishing. Chemoecology. Nutritional effects on chemical defense alter predator-prey dynamics.

Brückner Adrian, Heethoff Michael. © Springer International Publishing AG, part of Springer Nature 2018

Chemoecology (2018) 28:1–9
https://doi.org/10.1007/s00049-018-0253-9

CHEMOECOLOGY

ORIGINAL ARTICLE

Nutritional effects on chemical defense alter predator–prey dynamics

Adrian Brückner[1] · Michael Heethoff[1]

Received: 5 December 2017 / Accepted: 15 February 2018 / Published online: 26 February 2018
© Springer International Publishing AG, part of Springer Nature 2018

Abstract
Reservoir-based chemical defense (=reducible defense) is a widespread mechanism to repel predators in many invertebrates. We investigated the influence of macronutrients on the availability and regeneration of defensive secretions and parametrize a theoretical functional response model for reducible defense to predict nutritional effects on predator–prey dynamics. Our modeling approach showed that initially high amounts of defensive secretions provided an effective short-time defense, while higher regeneration rates were favorable under permanent predation pressure. Regeneration rates were correlated to the amount of dietary fat and carbohydrates, indicating an adaptive connection of macronutrients on chemical defense and predatory success—an effect covering two trophic levels. Our results underpin the urgent need to integrate dynamical aspects of chemical defense into the modeling of predator–prey interactions in food webs.

Keywords *Archegozetes longisetosus* · Theoretical ecology · Numerical model · Soil food web · Nutritional ecology

Introduction

Exocrine defensive chemicals influence many trophic interactions at all levels of biological organization (Berenbaum 1995; Eisner and Meinwald 1966; Pasteels et al. 1983). Hence, chemical defense is one of the most effective survival strategies in terrestrial ecosystems (see Eisner et al. 2005 for examples) that shapes complex predator–prey interactions among arthropods (e.g., Blum 1981; Eisner et al. 2005; Pasteels et al. 1983). Chemical defense is highly adaptive and beneficial for the defended individuals (Eisner 2003; Eisner et al. 2005; Eisner and Meinwald 1966; Glendinning 2007; Heethoff and Rall 2015) and defense may, therefore, be responsible for food web stability over longer time scales (e.g., Abrams and Walters 1996; Hammill et al. 2010).

In general, it has been assumed that de novo produced defensive allelochemicals are characteristic, species-specific and show limited intraspecific compositional variability (e.g., Pasteels et al. 1983; Raspotnig 2006a; Rothschild 1984; Whittaker and Feeny 1971). However, the allocation, efficiency and performance of chemical defense in arthropods seems to be more plastic (Berenbaum 1995; Blum 1981; Rothschild 1984) and there is growing evidence that various factors influence the quality and quantity of glandular exudates. The best known determinants changing qualitative and relative composition as well as the amounts of glandular chemicals are probably age/ontogenetic status (e.g., Blum 1996; Bodner and Raspotnig 2012; Brückner and Heethoff 2017), but also sex (e.g., Kullenberg et al. 1970; Unruh et al. 1998; Whitman et al. 1992), social caste (e.g., Goh et al. 1984; Prestwich 1979), bacterial symbionts (e.g., Becerra et al. 2015; Florez et al. 2015; Oliver et al. 2012), and especially diet (e.g., Holliday et al. 2009; Jones et al. 1986, 1987) can influence the degree of being chemically defended.

Environmental variations (i.e., carbon and nitrogen availability) cause changes in plant nutrient levels, which lead to changes of carbon/nitrogen-based secondary compounds (e.g., Bryant et al. 1989; Coley et al. 1985; Reichardt et al. 1991). In animals, two general mechanisms—sequestration and autogenous chemical defense—exist (Berenbaum 1995; Duffey 1980; Spencer 1988). Animals accumulating their

Communicated by Marko Rohlfs.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00049-018-0253-9) contains supplementary material, which is available to authorized users.

✉ Adrian Brückner
adrian.brueckner@gmail.com

✉ Michael Heethoff
heethoff@bio.tu-darmstadt.de

[1] Ecological Networks, Technische Universität Darmstadt, Schnittspahnstraße 3, 64287 Darmstadt, Germany

chemical defense via sequestration of host plants' or other animals' defensive chemicals are influenced by allocable dietary resources, since the access to distinct food sources determines the chemical composition and the repellent efficiency of defensive gland exudates (e.g., Duffey 1980; Jones et al. 1989; Saporito et al. 2009). However, the chemical defense of autogenously defended animals is also influenced by nutritional quality and diet breadth, because unbalanced/restricted diets may reduce the availability of precursor molecules for the de novo biosynthesis of defensive compounds (Jones et al. 1986, 1987, 1989; Whitman et al. 1992). Further, unbalanced diets can cause physiological stress (Simpson and Raubenheimer 2012) which might influence chemical defense as hypothesized by Jones et al. (1987, 1988). For example, in lubber grasshoppers and fungus beetles, different diets and artificially altered diet breadth caused differences in the qualitative (Jones et al. 1987) or relative composition (Holliday et al. 2009; Jones et al. 1986) and amount (Whitman et al. 1992) of de novo produced defensive chemicals.

To our best knowledge, however, no study so far has investigated the consequences of diet-dependent availability of defensive secretion amounts or related them to macronutrient quality of food. Here we use an experimentally derived functional response model that conceptualizes reservoir-based chemical defense ("reducible defense"; *sensu* Heethoff and Rall 2015). Using this model, we investigate (i) how changes in defensive chemistry caused by diet influence predator–prey dynamics and (ii) if macronutrients can explain outcomes of the model. To answer these questions, we parametrize the reducible defense functional response model with data derived from a feeding experiment using the autogenously defended oribatid mite *Archegozetes longisetosus* Aoki as a model organism.

Materials and method

Experimental setup and chemical analysis

The model organism *Archegozetes longisetosus* ran (Heethoff et al. 2007) is a parthenogenetic, opportunistically feeding oribatid mite from the tropics which autogenously produces a blend of hydrocarbons, terpenes and aromatic compounds that are released upon aggressors for defense (Heethoff and Raspotnig 2011; Heethoff et al. 2011). The mites do not selectively expel defensive compounds but use the whole blend of chemicals which are all bioactive and synergistically function as repellents (e.g., Raspotnig 2006b; Kuwahara 2010). *A. longisetosus* was reared at approximately 28 °C and 80–85% relative humidity in constant darkness on one out of ten resources for several generations (approximately 18 month). The ten different diets consisted of animal resources such as blood meal (blood; Common Baits, Rosenfeld, Germany) and bone meal (bone; Canina Pharma GmbH, Hamm, Germany); one bacterial resource, *Spirulina* powder (spirulina; Interaquaristik, Biedenkopf-Breidenstein, Germany); fungal resources, such as shiitake fungus powder (fungi; Arche Naturprodukte GmbH, Hilden, Germany) and grinded dry yeast (yeast; Rapunzel Naturkost GmbH, Legau, Germany); and a variety of herbal resources, such as *Chlorella* powder (chlorella; Naturya, Bath, UK), hemp protein powder (hemp; Naturya, Bath, UK), sweet lupine flour (lupine; Govinda Natur GmbH, Neuhofen, Germany), grinded mixed pollen grain (pollen; Ascopharm GmbH, Wernigerode, Germany) and wheat grass powder (wheat; Naturya, Bath, UK). Fresh food and water was provided ad libitum three times a week. For each resource, specimens were cultured in three separated plastic boxes ($100 \times 100 \times 50$ mm) grounded with 2 cm mixture of plaster of Paris/activated charcoal mixture (9:1).

At the start of the experiment, we treated 150 individuals per resource (= 1500 specimens in total) with an established hexane-recovery-hexane (HRH) protocol (Heethoff and Raspotnig 2012). This procedure does not influence the mites' life history parameters and leads to a complete depletion of defensive gland reservoirs (Heethoff and Raspotnig 2012), which we confirmed by control measurements of ten pooled individuals per resources (see "Materials and method" below). Afterwards, the remaining 1400 individuals were transferred into 30 new plastic boxes (again three boxes per resource and 40–50 individuals per box) and the mites were fed with the same food as they had received before the HRH treatment.

After 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 days (= 10 sampling dates), we randomly selected 3–4 mites from each culture box (= 10 individuals in total) and extracted their exocrine secretions by immersing them individually in 50 µl hexane (GC grade, 98%, Merck, Darmstadt, Germany) for 3 min. The extraction solvent also contained tetradecane (1 ng/µl; ≥ 99.8%, analytical standard, Sigma-Aldrich, Munich, Germany) as an internal standard. Crude extracts were stored at − 20 °C until GC/MS analyses. Individual mites were weighed with a microbalance after extraction (Mettler Toledo, XS3DU, Columbus, USA; with 0.1 µg readability and 1 µg repeatability).

The samples were analyzed with a QP 2010ultra GC/MS (Shimadzu, Duisburg, Germany). The GC was equipped with a ZB-5MS fused silica capillary column (30 m × 0.25 mm ID, $df = 0.25$ µm) from Phenomenex (Aschaffenburg, Germany) and 4 µl sample aliquots were injected in splitless mode and subsequently analyzed as described in Brückner and Heethoff (2017). The total ion currents of all detected compounds were summed up and the amount of the defensive secretions (ng) were calculated based on the internal

tetradecane standard as described in Brückner and Heethoff (2017).

Neutral lipid fatty acids (= fat), proteins/amino acids and C/N ratios of the ten resources were chemically characterized as described in (Brückner et al. 2017), using GC/MS, ion-exchange chromatography and gas chromatography, respectively. Data on the content of carbohydrates were extracted from the suppliers' information.

Modeling and statistics

The amounts of stored defensive secretions (= reservoir based chemical defense) are highly dynamic and increments of defensive secretions are repeatedly lost during attacks of predators until they drop under a quantitative threshold, and remaining amounts become too low to effectively repel a predator [= reducible defense, sensu Heethoff and Rall (2015)]. On the other hand, defensive chemicals are permanently synthesized and thus regenerated by exocrine glands (Heethoff 2012). Hence, the frequency and duration of interactions in a predator–prey system with reservoir-based chemical defense are mainly determined by the available amounts of defensive secretions, their regeneration rate and the predators' discovery rate. More detailed information on the model, terminology and further parameters can be found in Heethoff and Rall (2015). To estimate the maximum available amounts of defensive secretion S_{max} per individual (ng N^{-1}) of mites fed on different resources (i.e., regeneration performance), we used the mean of the three highest amounts of defensive secretions (ng N^{-1}) at a given time (Table 1). The differences of S_{max} (ng N^{-1}) among the resource treatments were assessed using a linear mixed-effect model after square root-transforming S_{max} (ng N^{-1}), to ensure normal distribution and variance homogeneity, with resource treatment as fixed and time as random factor (because we did not want to analyze the change over time in this model). Furthermore, we fitted the S_{max} (ng N^{-1}) secretion–regeneration over time data to Eq. 1 (with S_{max} as S_t and $S_{t=0} = 0$, confirmed by control measurements), using the generic nls()-function in R for each resource separately.

$$S_t = \frac{-K_S + K_s e^{\frac{R_S t}{K_S}} + S_{t=0}}{e^{\frac{R_S t}{K_S}}} \quad (1)$$

with K_S (ng N^{-1}) being the reservoir size (previously named "carrying capacity" in Heethoff and Rall 2015) and R_S (ng h^{-1} N^{-1}) being the regeneration rate of a subpopulation of defended prey. Equation 1 (Eq. 1) can hence be used to fit experimentally measured quantitative secretion data over time to estimate K_S and R_S for all ten resources.

Subsequently, K_S (ng N^{-1}) and R_S (ng h^{-1} N^{-1}) were used together with the discovery rates α (A_{area} h^{-1}) and handling times T_h (h N^{-1}) to parametrize the functional response model for each resource. The values of α (A_{area} h^{-1}) and T_h (h N^{-1}) were calculated based on Binzer et al. (2012) and Rall et al. (2012) using a predator body mass of 3.5 mg (corresponding to *Stenus juno*, Heethoff and Rall 2015) and measured prey body masses m_{mite} (µg) (mean of each resource; see Table 2) and the parameters given in Heethoff and Rall (2015). The success rate σ was set to 0.28 (see Heethoff et al. 2011) and the amount of defensive secretion lost per attack e_s (= transferring constant) was set to 50 ng N^{-1} (based on the information given in Raspotnig 2006b and amounts from; Heethoff 2012).

Since different diets influence not only chemical defense (e.g., Holliday et al. 2009; Whitman et al. 1992), but also the body mass of the prey (see "Results") and since the functional response also highly depends on the predator–prey body size ratio (e.g., Brose 2010; Kalinkat et al. 2013; Vucic-Pestic et al. 2010), we can only model the effects of resources as a combined functional response. We

Table 1 Regeneration data of the maximum defensive secretion S_{max} (ng N^{-1}) for each diet over time (h). Numbers represent the means of the three highest amounts of defensive secretions (ng N^{-1}) at a given time per diet

Defensive secretion after n days S_t (ng N^{-1})											
	$n_{day}=0$	$n_{day}=1$	$n_{day}=2$	$n_{day}=3$	$n_{day}=4$	$n_{day}=5$	$n_{day}=6$	$n_{day}=7$	$n_{day}=14$	$n_{day}=21$	$n_{day}=28$
Blood	0	0	0	0	0	0	0	0	0	0	0
Bone	0	3	14	12	50	120	115	165	163	114	200
Chlorella	0	3	5	21	23	19	30	58	32	100	89
Fungi	0	1	3	4	9	35	52	85	65	68	150
Hemp	0	3	7	37	61	72	115	161	172	135	171
Lupine	0	2	5	23	70	92	116	141	93	69	71
Pollen	0	2	10	9	28	31	43	116	119	112	120
Spirulina	0	24	77	48	171	175	238	241	256	198	292
Wheat	0	6	16	24	50	87	99	108	83	93	129
Yeast	0	29	82	70	130	108	190	286	261	315	247

Table 2 The model estimates (Eq. 1; mean ± SE) of the reservoir size K_S (ng N^{-1}) as well as the regeneration rate R_S (ng h^{-1} N^{-1}), the weighted body masses (mean ± SE) and statistics, the calculated discovery rates α (A_{area} h^{-1}) as well as handling times T_h (h N^{-1}), which were used to parametrize the predator–prey model (Heethoff and Rall 2015)

	Reservoir size K_S (ng N^{-1})				Regeneration rate R_S (ng h^{-1} N^{-1})				Body mass m_{mite} (µg)			Discovery rate α (A_{area} h^{-1})	Handling time T_h (h N^{-1})
	Estimate	SE	*t*	*P*	Estimate	SE	*t*	*P*	Mean	SE	Post hoc[§]		
Blood	0	0	–	–	0	0	–	–	63	3.5	d	0.126	0.385
Bone	177.5	33.1	5.37	*< 0.001*	25.1	7.6	3.29	*0.011*	82	7.4	bd	0.134	0.445
Chlorella	135.2	68.9	1.96	0.085	5.8	1.7	3.37	*0.009*	152	14.4	a	0.156	0.624
Fungi	258.4	301.9	0.86	0.417	6.4	2.4	2.67	*0.028*	80	8.3	bd	0.133	0.439
Hemp	173.4	24.9	6.96	*< 0.001*	24.6	5.8	4.26	*0.003*	105	4.9	b	0.143	0.509
Lupine	94.9	19.6	4.84	*0.001*	29.4	14.6	2.01	0.079	91	2.2	bd	0.138	0.471
Pollen	140.5	33.9	4.14	*0.003*	12.3	3.5	3.49	*0.008*	77	5.2	cd	0.132	0.429
Spirulina	263.7	29.2	9.04	*< 0.001*	56.5	12.4	4.55	*0.002*	104	6.7	abc	0.142	0.507
Wheat	112.5	15.3	7.36	*< 0.001*	21.4	5.5	3.91	*0.004*	73	1.4	d	0.130	0.417
Yeast	293.3	34.1	8.60	*< 0.001*	47.1	9.5	4.95	*0.001*	104	6.7	bd	0.142	0.507

Significant *P* values are denoted in italics

[§]Letters indicate homogenous groups (pairwise Mann–Whitney *U* tests with FDR correction)

were, however, interested in the functional consequences of dietary altered chemical defense and not in the combined effect. Therefore, we estimated the effective chemical defense as the number of surviving mite individuals caused by chemical defense Δ_{chem} ($N\ A_{area}{}^{-1}$) at a given time as:

$$\Delta_{chem} = N_{S,\,t_n} - N_{t_n}, \quad (2)$$

where $N_{S,\,t_n}$ ($N\ A_{area}{}^{-1}$) is the number of living, defended mites at a given time (i.e., model including chemical defense and body mass) and N_{t_n} ($N\ A_{area}{}^{-1}$) is the number of living, undefended mites at a given time (i.e., the null-model excluding chemical defense, but including body mass differences). We simulated both functional response scenarios according to Heethoff and Rall (2015) to calculate the effective chemical defense Δ_{chem} (Eq. 2) over time using the lsoda()-function with a total number of 100 prey individuals and a time step length of 0.1 h ($n_{steps} = 10{,}000$). The time of effective chemical protection t_{chem} (h) was estimated as the first time which applies to $\Delta_{chem} < 1$ ($N\ A_{area}{}^{-1}$).

The correlations between resource parameters (i.e., the amount of fatty acids, the amount of total protein, the amount of carbohydrates and the C/N ratio; see supplementary information table S2 for nutrient analyses), and reservoir size K_S (ng N^{-1}) as well as regeneration rate R_S (ng h^{-1} N^{-1}), were tested using Spearman's rank correlation and visualized as response surface plot. Furthermore, we tested the correlation between the effective chemical protection time t_{chem} (h) and K_S (ng N^{-1})/R_S (ng h^{-1} N^{-1}) using Spearman's rank. The differences in mite body masses m_{mite} (µg) grown on different resources were tested using a Kruskal–Wallis test with affiliated pairwise Mann–Whitney *U* tests. Type I error accumulation for all analyses was corrected with the false discovery rate correction (Benjamini and Hochberg 1995).

All statistical analyses were performed with R 3.3.1—"Bug in Your Hair" (R Core Team 2016), using the packages "deSolve" (Soetaert et al. 2010), "lme4" (Bates et al. 2015) and "MuMIn" (Barton 2016).

Results

We quantified the defensive gland secretions of mites ($n = 300$ individuals) feeding on ten different resources to measure the maximum amount of secretion S_{max} (ng N^{-1}) over a period of 28 days and to estimate the reservoir size K_S (ng N^{-1}) and the regeneration rate R_S (ng h^{-1} N^{-1}). Generally, we observed regeneration of defensive secretions for all resources, except for blood meal (Table 1). Additionally, the maximum amount of secretion S_{max} (ng N^{-1}) differed among the resource treatments (LMM $F_{9,90} = 43.97$, $P < 0.001$, $R^2_{LMM(m)} = 0.48$).

We fitted this chemical defense performance data (Table 1) as a non-linear relationship over time (Fig. 1) yielding nine regeneration curves. The estimated reservoir sizes K_S (ng N^{-1}) (Table 2) were significantly different for all resources except for chlorella ($P = 0.085$) and fungi ($P = 0.417$), while the estimated regeneration rates R_S (ng h^{-1} N^{-1}) (Table 2) showed a significant increase (slope) for all resources except lupine ($P = 0.079$). K_S as well as R_S were set as 0 for blood meal for all further analyses. The body masses m_{mite} (µg) were different among the experimental diet groups (Kruskal–Wallis: $N = 207$; $df = 9$; $\chi^2 = 75.38$,

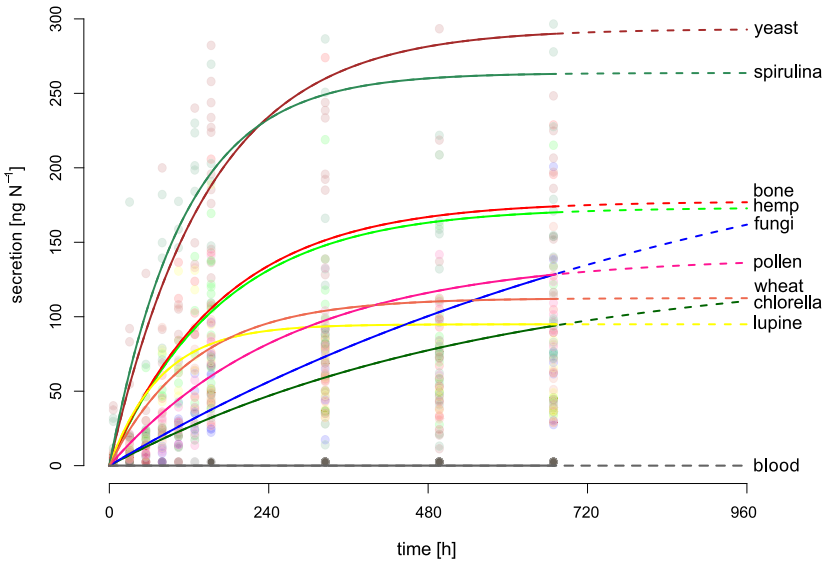


Fig. 1 Regeneration of defensive secretion S_{max} (ng N^{-1}) for each diet over time (h). Mites were experimentally depleted at $t=0$ h. Soil lines denote fitted experimental data, while dashed lines denote simulated values. Dots represent the raw data

$P<0.001$; detailed pairwise post hoc tests, see Table 2), and accordingly the body mass-dependent parameters handling time T_h (h N^{-1}) and discovery rate α (A_{area} h^{-1}) were different (Table 2). The reservoir sizes K_S (ng N^{-1}) were not correlated to any resource parameter (all $P>0.5$), while the regeneration rates R_S (ng h^{-1} N^{-1}) were positively correlated to the amount of fatty acids ($\rho_s=0.74$, $P=0.010$) and carbohydrates ($\rho_s=0.37$, $P=0.047$) in the diet, but not to the amount of protein or the C/N ratio (all $P>0.8$). Consequently, a high amount of fat together with a high proportion of carbohydrate in the food led to higher regeneration rates (Fig. 2).

We used all the results above to perform two separate types of functional response simulations. We simulated the number of living prey over time in (i) a scenario with initially undefended prey ($S_{max}=0$, for each resource) and (ii) a scenario with initially defended prey ($S_{max}=K_S$, for each resource), yielding ten exponential or s-shaped declining-curves for each resource (see supplement Fig S3). Based on these, we simulated the effective chemical protection Δ_{chem} ($N\,A_{area}{}^{-1}$) (Eq. 2), resulting in nine humped-shaped and one slope-less curve (Fig. 3) for the number mites surviving due to chemical protection over time. While mites fed with blood meal were not protected at all, especially fungi and chlorella mites were initially well-protected, but Δ_{chem} ($N\,A_{area}{}^{-1}$) quickly declined with time (Fig. 3). Initial Δ_{chem} ($N\,A_{area}{}^{-1}$) was intermediate for yeast and spirulina, but mites fed on these resources survived longer, hence showed a longer lasting effective chemical defense (Fig. 3). To further quantify these time effects, we defined the effective chemical protection time t_{chem} (h) as [$\Delta_{chem}<1$ ($N\,A_{area}{}^{-1}$)] and found about three times higher t_{chem} (h) for yeast and spirulina,

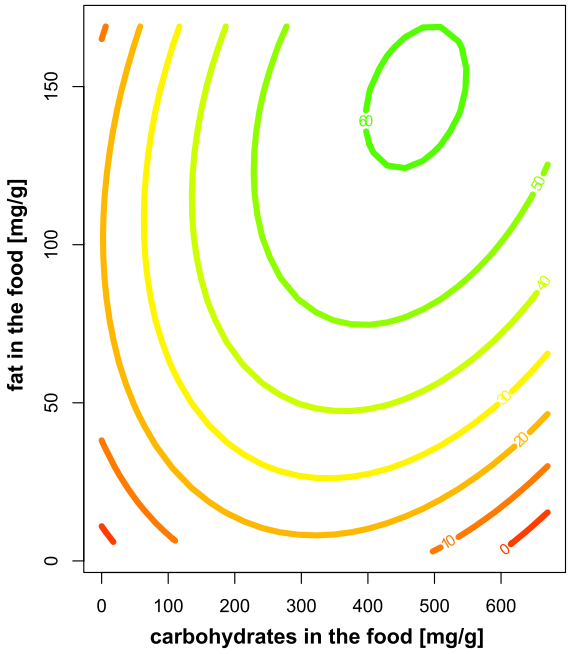


Fig. 2 Effects of dietary carbohydrates and fat on the regeneration rate of the defensive secretions. The response surface plot shows the regeneration rate across diet composition space. The colors correspond to the regeneration; red lines indicate a low regeneration rates, green colored lines represent high regeneration rates

compared to chlorella and fungi fed mites (see Table 3 for all resources). The effective chemical protection times t_{chem} (h) of the resources were correlated to the reservoir sizes

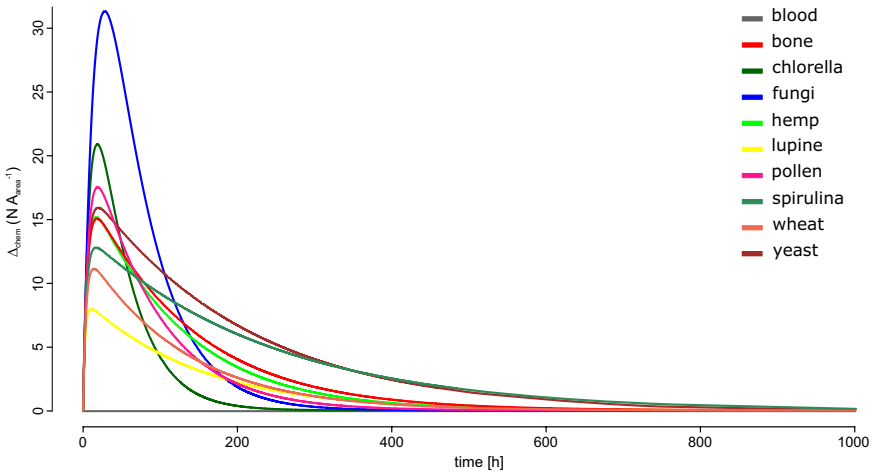


Fig. 3 Model simulations for the effective chemical protection Δ_{chem} ($N A_{area}^{-1}$) of mites reared on different diets within 1000 h

Table 3 The time of effective chemical protection t_{chem} (h) defined as $\Delta_{chem} < 1$ ($N A_{area}^{-1}$) for each diet

	t_{chem} (h)
Blood	0
Bone	378
Chlorella	158
Fungi	233
Hemp	338
Lupine	320
Pollen	258
Spirulina	608
Wheat	314
Yeast	568

K_S (ng N^{-1}) (Spearman's $\rho_s = 0.67$, $P = 0.033$) and to the regeneration rates R_S (ng h^{-1} N^{-1}) (Spearman's $\rho_s = 0.90$, $P < 0.001$).

Discussion

We investigated how different diets influence the dynamics of chemical defense by extending and parametrizing an already established predator–prey functional response model (Heethoff and Rall 2015) for chemical defense (see Fig. 4 for a conceptual overview). Generally, we found that diets with

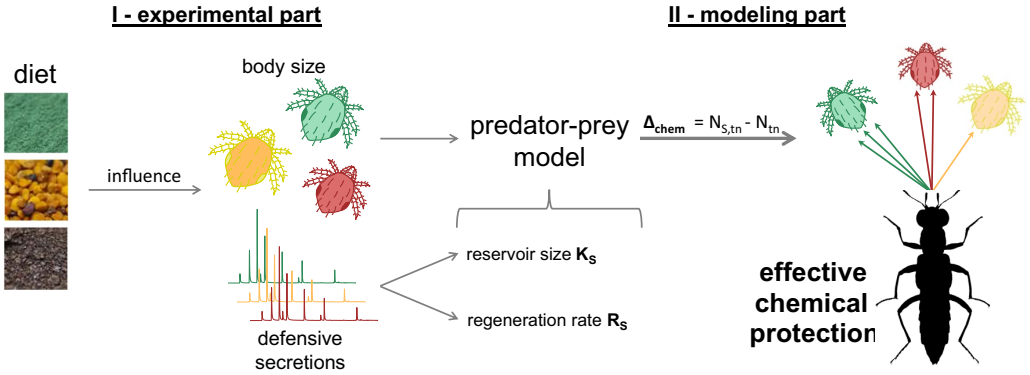


Fig. 4 Different diets influence the body size and defensive secretions of prey specimens (= mites). Body size shapes the general functional response of predator–prey interactions in common models, while differences in defensive secretions alter the "reducible chemical defense" (sensu Heethoff and Rall 2015) and therefore the dynamics of chemically mediated predator–prey interactions. Our extension of the original "reducible defense" model extracts the nutritional effects on chemical defense, ultimately yielding different effective chemical protection for prey fed on different resources

different compositions (see supplementary information S2) strongly influence the regeneration dynamics of defensive secretion S_t (ng N^{-1}) (Table 1; Fig. 1) and the body mass m_{mite} (µg) of the prey (Table 2). Hence, also the reservoir size K_S (ng N^{-1}) and the regeneration rate R_S (ng h^{-1} N^{-1}), as well as the discovery rate α (A_{area} h^{-1}) and the handling time T_h (h N^{-1}) changed (Table 2). Body mass and body mass-dependent parameters were influenced by diet (Table 2), but not directly related to resource composition. While K_S (ng N^{-1}) was not correlated to resource composition, R_S (ng h^{-1} N^{-1}) was positively correlated to the amount of fat and carbohydrates (Fig. 2), but not to the amount of proteins or the C/N of diets. The importance of lipids and carbohydrates for the biosynthesis seems also quite evident, considering that defensive secretions of *A. longisetosus* consist of three terpenes, six hydrocarbons and two aromatic aldehydes (see Heethoff and Raspotnig 2011 for details). All compounds are most likely synthesized de novo from acetyl-CoA (Morita et al. 2004; Shimizu et al. 2014), which can be produced from fatty acids via β-oxidation (Schulz 1991) and from carbohydrates via glycolysis and the TCA cycle (Sanders 2016). There seems to be no direct relationship between the nutritional balance (= C/N ratio) and the parameters K_S (ng N^{-1}) and R_S (ng h^{-1} N^{-1}). This result, along with the correlation to the amount of fat and carbohydrates allows to derive a hypothesis: a higher amount of biosynthetic energy equivalents—acetyl-CoA liberated from fats and sugars—enables a higher regeneration rates of defensive chemical in autonomously defended prey (hypothesis 1 = *energy – regeneration rate hypothesis*).

Our simulation results (Fig. 3) further showed that diet can influence the dynamics of reservoir-based, chemical defense in predator–prey interactions as well. The influence of nutritional quality on anti-predator defense is also known from other inducible defense-based systems found in plants or freshwater plankton (Bryant et al. 1989; Christjani 2015; Coley et al. 1985; Reichardt et al. 1991). For instance, Christjani (2015) showed that the strength of inducible morphological defense of *Daphnia pulex* Leydig (such called "neck–teeth") depends on algal food quality. Generally, the effective chemical protection Δ_{chem} ($N\ A_{area}{}^{-1}$) was higher in the beginning and decreased over time. The initial shapes of the curves were mostly determined by the per capita reservoir sizes K_S (ng N^{-1}), while the trends over time were mostly influenced by the regeneration rates R_S (ng h^{-1} N^{-1}). Hence, the effective chemical protection time t_{chem} (h) (Table 3), which we defined as the main performance parameter for a diet in our modeling approach, was higher correlated to R_S (ng h^{-1} N^{-1}) than to K_S (ng N^{-1}). Thus, the performance of chemical defense (see Fig. 3; Table 3) may follow a simple rule: in a scenario of constant (or high) predatory pressure, where the prey needs to "spend" a fixed amount of defensive secretion to successfully repel a predator, an initially high K_S provides (only) an effective short-time defense. Over time, however, it is more beneficial to have a higher R_S, because a constant availability of defensive secretion is needed to maintain an effective chemical protection (hypothesis 2 = *regeneration – effective chemical protection hypothesis*).

In summary, diets not only influence the chemical composition of defensive secretions in arthropods (e.g., Holliday et al. 2009; Jones et al. 1986, 1987; Whitman et al. 1992), but also can induce functional consequences related to the efficiency of chemical defense in predatory–prey interactions. Using a functional response model which mechanistically describes such chemically mediated predator–prey interactions, we derived two testable hypothesis—the energy-regeneration rate and the regeneration-effective chemical protection hypothesis—which lend themselves to further experimental evaluation. This brings attention to chemical defense as an important factor that can alter the dynamics, structure and stability of food webs.

Acknowledgements We thank Romina Schuster for experimental assistance and Björn C. Rall for discussing the model with us. AB was supported by the German Nation Academic Foundation (Studienstiftung des deutschen Volkes). This study was supported by the German Science Foundation (DFG; HE 4593/5-1).

Author contributions MH and AB designed the research; AB performed chemical analyses; AB analyzed and modeled the data; AB and MH wrote the paper. Both authors discussed and approved the final manuscript.

Compliance with ethical standards

Ethical statement There are no legal restrictions on working with mites.

Conflict of interest The authors declare no competing financial interests.

References

Abrams PA, Walters CJ (1996) Invulnerable prey and the paradox of enrichment. Ecology 77:1125–1133

Barton K (2016) MuMIn: multi-model inference. R package version 1156. https://CRAN.R-project.org/package=MuMIn

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Soft 67:1–48

Becerra JX, Venable GX, Saeidi V (2015) Wolbachia-free heteropterans do not produce defensive chemicals or alarm pheromones. J Chem Ecol 41:593–601

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate - a practical and powerful approach to multiple testing. J R Stat Soc B Methodol 57:289–300

Berenbaum MR (1995) The Chemistry of defense—theory and practice. P Natl Acad Sci USA 92:2–8

Binzer A, Guill C, Brose U, Rall BC (2012) The dynamics of food chains under climate change and nutrient enrichment. Philos Trans R Soc Lond B Biol Sci 367:2935–2944
Blum MS (1981) Chemical defenses of arthropods. Academic Press Inc., New York
Blum MS (1996) Semiochemical parsimony in the Arthropoda. Annu Rev Entomol 41:353–374
Bodner M, Raspotnig G (2012) Millipedes that smell like bugs: (E)-alkenals in the defensive secretion of the julid diplopod *Allajulus dicentrus*. J Chem Ecol 38:547–556
Brose U (2010) Body-mass constraints on foraging behaviour determine population and food-web dynamics. Func Ecol 24:28–34
Brückner A, Heethoff M (2017) The ontogeny of oil gland chemistry in the oribatid mite *Archegozetes longisetosus* Aoki (Oribatida, Trhypochthoniidae). Int J Acarol 43:337–342
Brückner A, Hilpert A, Heethoff M (2017) Biomarker function and nutritional stoichiometry of neutral lipid fatty acids and amino acids in oribatid mites. Soil Biol Biochem 115:35–43
Bryant JP, Kuropat PJ, Cooper SM, Frisby K, Owensmith N (1989) Resource availability hypothesis of plant antiherbivore defense tested in a South-African savanna ecosystem. Nature 340:227–229
Christjani M (2015) Chemical ecology of *Chaoborus* as predator and prey: Effects of infochemicals and food quality on inducible defences and gene expression in *Daphnia*. Dissertation, University of Cologne, Colonge
Coley PD, Bryant JP, Chapin FS (1985) Resource availability and plant antiherbivore defense. Science 230:895–899
Duffey SS (1980) Sequestration of plant natural-products by insects. Annu Rev Entomol 25:447–477
Eisner T (2003) For love of insects. Harvard University Press, Cambridge
Eisner T, Meinwald J (1966) Defensive secretions of arthropods. Science 153:1341–1350
Eisner T, Eisner M, Siegler M (2005) Secret weapons. Harvard University Press, Cambridge
Florez LV, Biedermann PHW, Engl T, Kaltenpoth M (2015) Defensive symbioses of animals with prokaryotic and eukaryotic microorganisms. Nat Prod Rep 32:904–936
Glendinning JI (2007) How do predators cope With chemically defended foods? Biol Bull 213:252–266
Goh SH, Chuah CH, Tho YP, Prestwich GD (1984) Extreme intraspecific chemical variability in soldier defense secretions of allopatric and sympatric colonies of *Longipeditermes longipes*. J Chem Ecol 10:929–944
Hammill E, Kratina P, Beckerman AP, Anholt BR (2010) Precise time interactions between behavioural and morphological defences. Oikos 119:494–499
Heethoff M (2012) Regeneration of complex oil-gland secretions and its importance for chemical defense in an oribatid mite. J Chem Ecol 38:1116–1123
Heethoff M, Rall BC (2015) Reducible defence: chemical protection alters the dynamics of predator–prey interactions. Chemoecology 25:53–61
Heethoff M, Raspotnig G (2011) Is 7-hydroxyphthalide a natural compound of oil gland secretions?—evidence from *Archegozetes longisetosus* (Acari, Oribatida). Acarologia 51:229–236
Heethoff M, Raspotnig G (2012) Triggering chemical defense in an oribatid mite using artificial stimuli. Exp Appl Acarol 56:287–295
Heethoff M, Laumann M, Bergmann P (2007) Adding to the reproductive biology of the parthenogenetic oribatid mite, *Archegozetes longisetosus* (Acari, Oribatida, Trhypochthoniidae). Turk J Zool 31:151–159
Heethoff M, Koerner L, Norton RA, Raspotnig G (2011) Tasty but protected-first evidence of chemical defense in oribatid mites. J Chem Ecol 37:1037–1043
Holliday AE, Walker FM, Brodie ED, Formica VA (2009) Differences in defensive volatiles of the forked fungus beetle, Bolitotherus cornutus, living on two species of fungus. J Chem Ecol 35:1302–1308
Jones CG, Hess TA, Whitman DW, Silk PJ, Blum MS (1986) Idiosyncratic variation in chemical defenses among individual generalist grasshoppers. J Chem Ecol 12:749–761
Jones CG, Hess TA, Whitman DW, Silk PJ, Blum MS (1987) Effects of diet breadth on autogenous chemical defense of a generalist grasshopper. J Chem Ecol 13:283–297
Jones CG, Whitman DW, Silk PJ, Blum MS (1988) Diet breadth and insect chemical defense: a generalist grasshopper and general hypotheses. In: Spencer KC (ed) Chemical mediation of coevolution. Academic Press Inc., San Diego, pp 447–476
Jones CG, Whitman DW, Compton SJ, Silk PJ, Blum MS (1989) Reduction in diet breadth results in sequestration of plant-chemicals and increases efficacy of chemical defense in a generalist grasshopper. J Chem Ecol 15:1811–1822
Kalinkat G, Schneider FD, Digel C, Guill C, Rall BC, Brose U (2013) Body masses, functional responses and predator-prey stability. Ecol Lett 16:1126–1134
Kullenberg B, Bergstrom G, Stallberg-Stenhagen S (1970) Volatile components of the cephalic marking secretion of male bumble bees. Acta Chem Scand 24:1481–1485
Kuwahara Y (2010) How astigmatic mites control the emission of two or even three types of pheromones from the same gland. In: Sabelis M, Bruin J (eds) Trends in acarology. Springer, Dordrecht, pp 241–247
Morita A, Mori N, Nishida R, Hirai N, Kuwahara Y (2004) Neral (the alarm pheromone) biosynthesis via the mevalonate pathway, evidenced by D-glucose-1-^{13}C feeding in *Carpoglyphus lactis* and ^{13}C incorporation into other opisthonotal gland exudates. J Pest Sci 29:27–32
Oliver KM, Noge K, Huang EM, Campos JM, Becerra JX, Hunter MS (2012) Parasitic wasp responses to symbiont-based defense in aphids. BMC Biol 10:11
Pasteels JM, Gregoire JC, Rowellrahier M (1983) The chemical ecology of defense in arthropods. Annu Rev Entomol 28:263–289
Prestwich GD (1979) Chemical defense by termite soldiers. J Chem Ecol 5:459–480
R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria ISBN 3-900051-07-0, http://www.R-project.org
Rall BC, Brose U, Hartvig M, Kalinkat G, Schwarzmuller F, Vucic-Pestic O, Petchey OL (2012) Universal temperature and body-mass scaling of feeding rates. Philos Trans R Soc Lond B Biol Sci 367:2923–2934
Raspotnig G (2006a) Characterisation of monophyletic oribatid groups by oil gland chemistry—a novel systematic approach in Oribatida (Acari). Abh Ber Naturkundemus Görlitz 78:31–46
Raspotnig G (2006b) Chemical alarm and defence in the oribatid mite *Collohmannia gigantea* (Acari: Oribatida). Exp Appl Acarol 39:177–194
Reichardt PB, Chapin FS, Bryant JP, Mattes BR, Clausen TP (1991) Carbon nutrient balance as a predictor of plant defense in Alaskan balsam poplar—potential importance of metabolite turnover. Oecologia 88:401–406
Rothschild M (1984) Citation classic: secondary plant-substances and warning coloration in insects. Life Sci 17:289
Sanders LM (2016) Carbohydrate: digestion, absorption and metabolism. In: Caballero B, Finglas PM, Toldrá F (eds) Encyclopedia of food and health. Academic Press, Oxford, pp 643–650
Saporito RA, Spande TF, Garraffo HM, Donnelly MA (2009) Arthropod alkaloids in poison frogs: a review of the dietary hypothesis. Heterocycles 79:277–297

Schulz H (1991) Beta-oxidation of fatty acids. Biochim Biophys Acta 1081:109–120

Shimizu N, Naito M, Mori N, Kuwahara Y (2014) De novo biosynthesis of linoleic acid and its conversion to the hydrocarbon (*Z,Z*)-6,9-heptadecadiene in the astigmatid mite, *Carpoglyphus lactis*: incorporation experiments with ^{13}C-labeled glucose. Insect Biochem Mol Biol 45:51–57

Simpson SJ, Raubenheimer D (2012) The nature of nutrition. Princeton University Press, Princeton

Soetaert K, Petzoldt T, Setzer RW (2010) Solving differential equations in R: package deSolve. J Stat Soft 33:1–25

Spencer KC (1988) Chemical mediation of coevolution. Academic Press Inc., San Diego

Unruh LM, Xu RD, Kramer KJ (1998) Benzoquinone levels as a function of age and gender of the red flour beetle, *Tribolium castaneum*. Insect Biochem Mol Biol 28:969–977

Vucic-Pestic O, Rall BC, Kalinkat G, Brose U (2010) Allometric functional response model: body masses constrain interaction strengths. J Anim Ecol 79:249–256

Whitman DW, Jones CG, Blum MS (1992) Defensive secretion production in lubber grasshoppers (Orthoptera, Romaleidae)—influence of age, sex, diet, and discharge frequency. Ann Entomol Soc Am 85:96–102

Whittaker RH, Feeny PP (1971) Allelochemics–chemical interactions between species. Science 171:757–770

10.3 Biochemistry

10.3.1 Publication 5: **Biomarker function and nutritional stoichiometry of neutral lipid fatty acids and amino acids in oribatid mites**

Adrian Brückner, Andrea Hilpert and Michael Heethoff

Published in: Soil Biology and Biochemistry 115: 35-43

Submitted: 25.04.2017, Accepted: 19.07.2017, Published: 22.10.2017

Authors contributions:

AB and MH designed the research; AB performed the experiment, AB and AH performed chemical analyses; AB analyzed the data; AB and MH wrote the paper. All authors discussed and approved the final manuscript.

Reprinted by permission from Elsevier. Soil Biology & Biochemistry. Biomarker function and nutritional stoichiometry of neutral lipid fatty acids and amino acids in oribatid mites. Brückner Adrian, Hilpert Andera, Heethoff Michael. © Elsevier Ltd. 201

Soil Biology & Biochemistry 115 (2017) 35–43

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Biomarker function and nutritional stoichiometry of neutral lipid fatty acids and amino acids in oribatid mites

Adrian Brückner*, Andrea Hilpert, Michael Heethoff**

Ecological Networks, Department of Biology, Technische Universität Darmstadt, Schnittspahnstraße 3, 64287 Darmstadt, Germany

ARTICLE INFO

Article history:
Received 25 April 2017
Received in revised form
11 July 2017
Accepted 19 July 2017

Keywords:
Dietary routing
Proteins
Lipids
Oribatida
Trophic ecology
Biochemical ecology

ABSTRACT

Biomarkers (e.g. fatty acids, amino acids, stable isotopes, and molecular barcodes) have become increasingly important for investigating food web structure and nutrient flow in soil ecosystems. While the biomarker function of fatty acids has been investigated for some soil animal taxa (e.g. collembolans and nematodes), their role in soil-dwelling oribatid mites remained unknown. Here, we investigate the biomarker function and nutritional stoichiometry of neutral lipid fatty acids (NLFA) and amino acids in oribatid mites. We reared the opportunistic model oribatid mite species *Archegozetes longisetosus* on ten different resources of animal, bacterial, fungal and herbal origin. We analyzed the neutral lipid fatty acid and amino acid compositions of resources and consumers with gas chromatography/mass spectrometry (GC/MS) and ion-exchange chromatography (IEC), respectively. We found diet-dependent amounts and compositions of NLFA in the oribatid mites, but amino acids were stable and independent of diet. Consumer NLFA composition could be used as a reliable predictor of diet using data mining approaches (i.e., Random Forest), while amino acid profiles reflected diet-independent intrinsic physiological properties and confirm the homeostatic protein stoichiometry hypothesis for oribatid mites.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Understanding food web structure, food sources and resource specialization is fundamental to reveal multichannel feeding, reticulated organization and flow of soil organic matter as well as to understand the enigmatically high diversity of soil animals in terrestrial ecosystems (Anderson, 1975; Scheu and Setälä, 2002; Wolkovich, 2016). The role of microarthropods as important participants structuring the soil, e.g. the formation of soil aggregates via the high production of fecal- and necrobiomass or the degradation of soil structure caused by high local abundances, as well as contributing to energy and material flow (e.g. carbon and nitrogen) has received increasing attention (e.g. Scheu et al., 2005; Maaß et al., 2015; Soong and Nielsen, 2016). To clarify soil microarthropods' functional role as decomposers, scavengers and potentially predators, biomarker techniques like molecular gut barcoding (e.g. Juen and Traugott, 2006), stable isotope ratios (e.g. Scheu and Falca, 2000; Pollierer et al., 2009; Maraun et al., 2011) and fatty acid profiles (Ruess and Chamberlain, 2010 and references therein) have been used.

Among soil microarthropods, especially the ubiquitous, diverse and particle-feeding acarine order Oribatida is of particular interest to draw a more conclusive picture of resource-consumer interactions in soils (Norton, 2007; Wehner et al., 2016). While only some pioneer studies on gut barcoding are available (Remén et al., 2010; Heidemann et al., 2011), oribatid mites represent a group which has been continuously examined regarding stables isotopes, providing information about food niche differentiation, trophic food web structure and isotopic fragmentation (e.g. Schneider et al., 2004; Maraun et al., 2011; Heethoff and Scheu, 2016). Stable isotopes have helped to understand general food web structure regarding oribatid mites, but failed to identify distinct food sources (Maraun et al., 2011). Therefore, the use of biomarker molecules, such as amino acids for nitrogen and fatty acids (e.g., neutral lipid fatty acids, NLFA) for carbon may provide more in-depth information about their feeding ecology.

While trophic transfer of potential biomarker fatty acids has been studied intensively in other soil animal taxa, such as collembolans, nematodes or centipedes (e.g. Ruess et al., 2002; Chamberlain et al., 2004, 2005; Ruess et al., 2005; Haubert et al., 2006; Pollierer et al., 2010), there are no studies testing

* Corresponding author.
** Corresponding author.
E-mail addresses: adrian.brueckner@gmail.com (A. Brückner), heethoff@bio.tu-darmstadt.de (M. Heethoff).

http://dx.doi.org/10.1016/j.soilbio.2017.07.020
0038-0717/© 2017 Elsevier Ltd. All rights reserved.

incorporation of fatty acids into body lipids of oribatid mites. Only one study considered oribatids in a broad carbon flux field experiment using stable isotopes of fatty acids (Pollierer et al., 2012), providing information about energy and carbon flow in a below ground food web. However, this setup was inadequate to show if and how resource-fats were transferred into oribatid mites, because analyses of field-collected animals do not provide information on dietary routing and biomarker reliability (Chamberlain et al., 2005). Beside fatty acids, there is only one short note about amino acids in oribatid mites (Butler and Tonn, 1963) - and no information about trophic transfer and nutritional stoichiometry of amino acids are available at all. Therefore, one of the main organic-nitrogen storage forms in soils (e.g. Jones and Kielland, 2002; Jones et al., 2002) with a potential biomarker function has been ignored. For the direct stoichiometric transfer of amino acid signals from resources to consumers, Anderson et al. (2004) assumed that homeostasis of body protein (and therefore amino acid composition) may be a highly conserved pattern, because cellular proteins are similar in each individual of one species, since they are based on the same genetic information, and should not differ because of different diets. On the other hand, however, there is some evidence, from microbial and marine systems showing that amino acids may also possess biomarker function (Boschker and Middelburg, 2002; Arthur et al., 2014). Hence, more controlled studies about protein/amino acid stoichiometry and composition in different animal taxa are needed to test the validity of these hypotheses.

Based on the need of more laboratory experiments regarding biomarker reliability and nutrient stoichiometry (e.g. Anderson et al., 2004; Chamberlain et al., 2005; Maraun et al., 2011), we set up a no-choice feeding experiment using the parthenogenetic model oribatid mite species *Archegozetes longesitosus* Aoki (Heethoff et al., 2013). *A. longisetosus* is an opportunistic particle feeder and culturable on very different resources (see Heethoff and Scheu, 2016). We used a broad spectrum of ten resources of animal, bacterial, fungal and herbal (algae, seeds, pollen and vegetative tissues) origin. We used gas-chromatography/mass-spectrometry (GC/MS) and ion exchange chromatography to analyze patters of fatty acids and amino acids, respectively, in both resources and oribatid mites. We aimed to answer the following questions: i) are neutral lipid fatty acids (NLFA) reliable nutritional biomarkers for oribatid mites? and ii) are amino acids also suitable as trophic markers or are they in homeostasis?

2. Materials and methods

2.1. Experimental design

Archegozetes longesitosus ran (Heethoff et al., 2007; hereafter "mites") were reared at approx. 28 °C and 80–85% relative humidity in constant darkness. The mites were fed three times a week with: animal resources such as blood meal (blood; Common Baits, Rosenfeld, Germany) and bone meal (bone; Canina Pharma GmbH, Hamm, Germany); one bacterial resource, *Spirulina* sp. powder (spirulina; Interaquaristik, Biedenkopf-Breidenstein, Germany); fungal resources, such as shiitake fungus, *Lentinula edodes* powder (fungi; Arche Naturprodukte GmbH, Hilden, Germany) and grinded dry yeast, *Saccharomyces cerevisiae* (yeast; Rapunzel Naturkost GmbH, Legau, Germany); and a variety of herbal resources, such as *Chlorella* sp. powder (chlorella; Naturya, Bath, UK), hemp, *Cannabis sativa* protein powder (hemp; Naturya, Bath, UK), sweet lupine, *Lupinus albus* flour (lupine; Govinda Natur GmbH, Neuhofen, Germany), grinded mixed pollen grain (pollen; Ascopharm GmbH, Wernigerode, Germany) and wheat grass, *Triticum* sp. powder (wheat; Naturya, Bath, UK). The food was provided *ad libitum* as recommended by Raubenheimer and Simpson (1997) for nutritional transfer experiments. For each resource, specimens were cultured in three separated plastic boxes (100 × 100 × 50 mm) grounded with 2 cm mixture of plaster of Paris/activated charcoal (9:1) for 11 month and numerous generations (where the mites exclusively fed on one of the resources) prior to the analysis. For the measurements, 60 (±15, depending on size) mite individuals were randomly removed from each of the 30 culturing containers and transferred into ten new boxes (one for each resource). The mites were kept in these boxes without food for 3 days. Then, 60 ± 15 individuals with an empty alimentary tract (visual inspection) were randomly taken from these boxes and frozen at −28 °C until chemical analysis. This procedure was replicated with new mites three times at intervals of two weeks.

2.2. Fatty acid analysis

Total lipids were extracted from the resource powders and mites (4–7 mg) using 1 mL of chloroform:methanol, 2:1 (V/V) according to Folch et al. (1957) over a period of 24 h. Animals were directly refrozen after the extraction and subsequently dried, weighted with a microbalance (Mettler Toledo, XS3DU, Columbus, USA; with 0.1 μg readability and 1 μg repeatability) and stored until amino acids analyses. Extracts were purified and separated according to the method described by Frostegård et al. (1991). Briefly: SiOH-columns (Chromabond® SiOH, Macherey-NagelGmbH & Co. KG, Düren, Germany) were washed and conditioned with 6 mL hexane. Subsequently, samples were applied on the column and elution of neutral lipids was accomplished with 4 mL chloroform. Afterwards the chloroform fractions were evaporated to dryness under gentle nitrogen gas flow and residuals were redissolved in 200 μL dichloromethane:methanol, 2:1 (V/V). 100 μL for animals and 20 μL for resources were transferred to new glass vials with a conical inlet (150 μL) and 20 μL of internal standard (C19:0 in methanol; 220 ng/μL) were added and evaporated to dryness again. Subsequently samples were derivatized to fatty acid methyl esters (FAMEs) with 20 μL TMSH (trimethylsulfonium hydroxide; 0.25 M in MeOH from Fluka, Sigma-Aldrich, St. Louis, USA) reagent. TMSH is a methylation agent which directly transesterifies free fatty acids or lipids such as triglycerids to the corresponding FAMEs. TMSH in methanol can be directly injected and residual non-FAME compounds pyrolyze within the injector. All solvents were analytical grade chemicals for gas chromatography (>99.8% purity) purchased from Merck KGaA, Darmstadt, Germany.

FAME samples of neutral lipid fatty acids (NLFAs) were analyzed with a QP2010 Ultra GC/MS (Shimadzu, Duisburg, Germany). The gas chromatograph (GC) was equipped with a ZB-5MS fused silica capillary column (30 m × 0.25 mm ID, df = 0.25 μm) from Phenomenex (Aschaffenburg, Germany). One microliter sample aliquots were injected by using an AOC-20i autosampler-system from Shimadzu, Duisburg, Germany into a programmable temperature vaporizing-split/splitless-injector (Optic 4, ATAS GL, Eindhoven, Netherlands), which operated in splitless-mode. The injection-temperature was 70 °C (5 s hold) which was raised to 300 °C with a heating rate of 30 °C sec^{-1} and then an isothermal hold for 59 min. Hydrogen was used as carrier-gas with a constant flow rate of 2.89 mL/min. The temperature of the GC oven was raised from initial 60 °C for 1 min, to 150 °C with a heating-rate of 15 °C min^{-1}, to 260 °C with a heating-rate of 3 °C min^{-1}, to 320 °C with a heating-rate of 10 °C min^{-1} and then an isothermal hold at 320 °C for 10 min. Electron ionization mass spectra were recorded at 70 eV from m/z 40 to 650. The ion source of the mass spectrometer and the transfer line were kept at 250 °C.

Methyl esters of the NLFAs (hereafter "fatty acids") were identified by comparing gas chromatographic retention times and mass spectrometric fragmentation patters with those of the Supelco® 37

Component FAME Mix standard and the Bacterial Acid Methyl Ester (BAME) Mix standard as well as commercially available fatty acids (all Sigma-Aldrich, St. Lois, USA) and published literature data (Zelles et al., 1992; Buse et al., 2013; Stein, 2015). The identity and double bond positions of C16:2Δ9,12, C16:3Δ9,12,15 and C18:3Δ6,9,12 were additionally checked by iodine catalyzed dimethyl disulfide derivatization (Dunkelblum et al., 1985). The configurations of the double bonds were not specifically determined.

2.3. Amino acid analysis

For amino acid analyses, dried mite samples (all three replicates from the fatty acid analyses) were pooled to achieve enough material. Approximately 5 mg or 2–5 mg dried food powder or animals, respectively, were hydrolyzed by diluting in 200 μL of hydrochloric acid (6 mol L^{-1}) and boiling for 4 h at 100 °C. Afterwards, residuals were cooled to room temperature, centrifuged (10 min at 14,800 rpm) and the supernatants were transferred into fresh tubes and were evaporated to dryness at 100 °C. Dried samples were redissolved in 200 μL of deionized water and evaporated. Samples subsequently were redissolved again in 200 μL of deionized water and further processed as described in Leonhardt and Blüthgen (2012). Finally, 20 μL of hydrolyzed amino acid samples were measured with an ion-exchange chromatograph (IEC) with ninhydrin post-column derivatization (Biochrom 20+, Amino Acid Analyzer, Cambridge, UK). Elution on an Ultropac-8-ion-exchange-column was accomplished with five different lithium-buffer solutions (pH range: 2.8–3.55), a solvent flow rate of 25 mL min^{-1} and a temperature gradient from 32 to 75 °C over a period of approx. 2.5 h

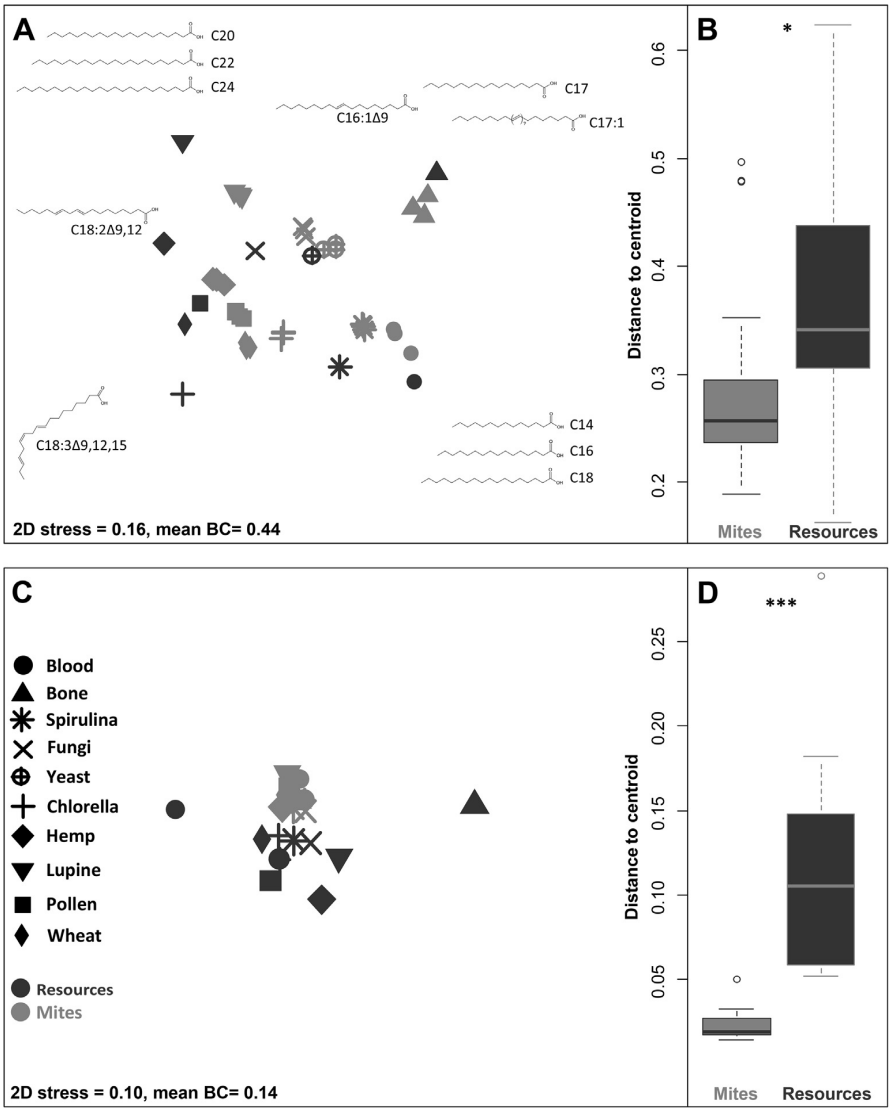


Fig. 1. Fatty acid (A–B) and amino acid (C–D) patters of mites that fed on different resources. A: nMDS plot of neutral lipid fatty acid patterns of mites (light grey) and resources (dark grey). C: nMDS plot of amino acid patters of mites (light grey) and resources (dark grey). B + D: Multivariate dispersion (= group variance) of mite and resources patters measured as the individual point's distance to the groups' centroid. Different symbols correspond to resource and mites fed on this resource. Shown fatty acids (A) are significantly contributing compounds ($p < 0.01$). PERMDISP: *$p < 0.05$; ***$p < 0.001$.

Table 1
Neutral lipid fatty acids (NLFA in %) of the resources and mites fed on the different resources as well as the amount of total NLFAs and proportion of unsaturated NLFA.

	Resource										Mite									
	Blood	Bone	Chlor	Fungi	Hemp	Lupine	Pollen	Spiru	Wheat	Yeast	Blood	Bone	Chlor	Fungi	Hemp	Lupine	Pollen	Spiru	Wheat	Yeast
C12:0	1.6	0.2	0.1	0.2	<0.1	<0.1	1.3	0.2	0.6	0.3	0.7	0.3	0.4	0.6	0.3	0.2	0.6	0.5	0.5	0.2
C14:0	3.8	1.8	0.6	0.8	0.1	0.1	0.9	0.5	2.1	0.8	1.2	1.0	0.9	0.8	0.5	0.3	0.8	1.1	0.8	0.4
iC15:0	–	0.1	0.1	–	–	–	–	–	–	–	–	<0.1	0.2	–	–	–	–	–	–	–
C15:0	–	0.1	–	0.4	–	–	–	–	–	–	–	<0.1	–	0.2	–	–	–	–	–	–
C16:0	46.1	34.8	38.3	17.6	10.8	6.4	36.7	71.8	31.2	24.9	48.2	27.8	37.4	13.2	13.1	8.4	24.1	45.3	23.2	13.8
iC17:0	–	0.3	–	–	–	–	–	–	–	–	–	<0.1	–	–	–	–	–	–	–	–
C17:0	–	0.4	0.3	0.3	–	–	–	–	–	0.2	–	0.2	0.5	1.0	–	–	–	–	–	0.4
C18:0	40.6	16.9	9.5	9.7	5.5	2.5	6.2	5.5	14.2	10.8	38.5	26.1	39.1	29.7	26.3	12.6	26.3	33.3	37.4	26.6
C20:0	–	–	–	–	0.4	0.5	0.2	–	0.2	0.2	–	–	<0.1	0.3	0.1	0.2	0.1	–	0.1	0.1
C22:0	–	–	<0.1	–	0.1	1.2	0.2	–	0.2	–	–	–	–	–	<0.1	0.4	0.1	–	<0.1	–
C24:0	–	–	<0.1	–	<0.1	0.1	0.1	–	0.1	–	–	–	–	–	<0.1	<0.1	<0.1	–	–	–
C14:1Δ9	–	0.1	–	–	–	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–
C16:1Δ9	–	1.8	0.9	0.2	–	<0.1	–	3.8	–	10.7	–	0.7	0.1	0.1	–	0.1	–	0.4	–	4.0
C17:1[a]	–	0.2	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–
C18:1Δ9	6.5	40.6	–	–	–	70.6	–	1.6	–	22.6	8.0	41.4	–	25.4	–	62.3	–	10.8	–	38.6
C18:1Δ9+ C18:3Δ9,12,15[b]	–	–	10.1	23.2	19.1	–	22.5	–	36.4	–	–	–	12.6	–	22.7	–	15.4	–	28.2	–
C18:1Δ11	–	2.5	0.7	–	–	–	0.6	–	–	0.7	0.2	0.7	0.2	–	–	–	0.4	–	–	1.4
C20:1Δ11	–	–	–	–	0.1	2.5	–	–	–	–	–	–	–	–	<0.1	0.4	–	–	–	–
C22:1[a]	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	<0.1	–	–	–	–
C16:2Δ9,12	–	–	6.6	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–
C18:2Δ9,12	1.5	0.2	16.5	38.0	54.6	13.6	20.0	13.3	4.6	25.8	3.2	1.4	6.9	26.6	32.9	13.8	23.7	7.4	6.3	13.0
C18:2[a]	–	–	1.3	9.4	9.1	2.0	1.6	1.6	–	2.9	–	–	0.4	2.3	3.9	1.4	1.3	1.2	0.5	1.5
C16:3Δ9,12,15	–	–	5.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C18:3Δ6,9,12	–	–	9.8	–	–	0.1	9.8	1.8	10.6	–	–	–	0.1	–	–	–	7.2	–	3.1	–
Unsaturated NLFAs [%]	8.0	45.4	50.9	70.8	82.9	89.2	54.5	22.1	51.6	62.7	11.4	44.4	20.4	54.4	59.6	78.1	48.0	19.8	38.1	58.5
Amount [μg/mg]	3	91	30	19	122	169	33	42	12	23	50	74	35	77	56	123	56	30	37	34

a Position of the double bond(s) not determined.

b Peaks of oleic acid (C18:1Δ9) and α-linolenic acid (C18:3Δ9,12,15) were chromatographically not well separated, hence we analyzed the area of the peak range, if C18:3Δ9,12,15was present.

according to the supplier's information. A standard amino acid mixture (200 μmol L^{-1} per amino acid; Laborservice Onken GmbH, Gründau, Germany) was used as external standard.

2.4. Data processing and statistical analysis

Compositional data of amino- and fatty acid profiles were ordinated as Bray-Curtis similarities and non-metric multidimensional scaling (nMDS) plots using metaMDS implemented in the R package "vegan" (Oksanen et al., 2016). Additionally, PERMANOVA and PERMDIPS were used to test for compositional and dispersion differences between resources and mites (amino- and fatty acids) or among the different treatment groups (fatty acids). Furthermore, Random Forests (Breiman, 2001) were used to test whether different *a priori* defined groups of animals (resources and resource types) could be correctly assigned to their diet based on their chemical profiles. For PERMANOVA and Random Forest 10,000 permutations were performed and for Random Forest the number of randomly sampled variables was set to mtry = 5 (with mtry = sqrt(p); where p is number of fatty acids). These statistical analyses were performed with R version 3.2.1 "World-Famous Astronaut" (R Core Development Team, 2015). For further information and a detailed R script see Brückner and Heethoff (2017). Amount data of amino- and fatty acids were standardized using the dry weight of resources and animals. Subsequently, correlation analyses of the amounts of amino- and fatty acids of resources and animals were performed using Pearson's r test based on means in PAST 3 (Hammer et al., 2001).

3. Results

3.1. Fatty acid composition of resources and mites

Fatty acid compositions of the ten resources were very different (Fig. 1A, Table 1). Correspondingly, the compositional dispersion within resources was higher (mean distance = 0.36, Fig. 1B) than within mites (mean distance = 0.28; Fig. 1B), and this difference was significant (PERMDISP: $F_{1,38} = 5.4$, $p = 0.032$). Furthermore, the fatty acid compositions of the mites, reared on the ten different resources, differed significantly (PERMANOVA: $pseudoF_{9,20} = 222.5$, $R^2 = 0.99$, $p < 0.001$; Fig. 1A, Table 1). Resources and mites differed from each other based on their fatty acid composition (PERMANOVA: $pseudoF_{1,38} = 4.0$, $R^2 = 0.09$, $p = 0.012$), although both groups were not clearly distinct (Fig. 1A). The resources blood and spirulina were mainly characterized by C16:0 and C18:0, while plant derived food exclusively showed the longer chained fatty acids C20:0, C22:0 and C24:0 (Table 1). Especially lupine had a very high proportion of C18:1Δ9 (70.6%), which to a lesser extend also characterized bone (40.6%) together with iC15:0, C15:0, iC17:0 and C17:0. Furthermore, fungal resources, but also hemp and pollen, had high percentages of C18:2Δ9, 12. Yeast had an exclusively high proportion of C16:1Δ9 and wheat showed a comparatively high proportion of the C18:1Δ9/C18:3Δ9,12,15 mixture as well as 18:3Δ6,9,12 (which was also found in chlorella and pollen). Overall, the patterns observed in resources were similar to those of the mites (Table 1), and the ratio of unsaturated fatty acids in resources and mites was highly correlated (Pearson's $r = 0.91$, $p < 0.001$). Yet, the amounts of some fatty acids were lower in mites compared to the resource. The ratio of C18:1Δ9 to C18:2Δ9,12 for mites (or food) from different origins was 10.7 (food: 21.3) for animal origin, 4.5 (food: 4.2) for plant origin, 1.6 (food: 3.1) for fungal and 1.6 (food: 0.7) for bacterial origin. We also tested whether fatty acid profiles of mites could be used to assign them to their diet. For both selected grouping possibilities, the ten resources and more generalized

Table 2
Amino acids (AS in %) of the resources and mites fed on the different resources as well as the total amount of amino acids.

	Resource										Mite									
	Blood	Bone	Chlor	Fungi	Hemp	Lupine	Pollen	Spiru	Wheat	Yeast	Blood	Bone	Chlor	Fungi	Hemp	Lupine	Pollen	Spiru	Wheat	Yeast
Asp	12.4	5.9	10.4	10.4	12.3	13.1	10.6	10.8	11.8	11.7	9.9	10.3	10.4	10.4	10.6	9.8	10.2	10.7	10.4	10.4
OH-Pro	–	8.4	–	–	–	–	0.6	–	–	–	–	2.2	–	–	–	–	–	–	–	–
Thr	3.2	2.5	4.9	5.4	3.1	3.5	3.3	5.2	4.6	4.6	3.6	4.2	4.2	4.1	4.2	4.3	4.3	4.1	4.3	4.5
Ser	6.9	4.4	6.1	8.4	7.8	7.8	6.3	7.5	6.4	7.4	6.5	6.8	7.0	7.2	7.4	6.8	7.1	7.0	7.3	7.2
Glu	8.1	9.2	10.5	20.0	17.5	20.8	8.1	12.5	10.4	14.8	10.9	10.2	10.0	10.9	11.0	10.7	10.5	10.3	10.9	10.6
Pro	4.9	10.7	6.7	5.3	5.8	5.8	24.8	4.8	6.9	7.0	5.6	5.7	6.1	6.4	5.8	6.0	5.9	5.6	5.9	5.5
Gly	10.3	27.4	12.1	9.4	11.3	4.5	9.2	10.6	13.4	10.6	18.5	17.0	17.3	17.7	17.0	16.4	15.5	17.7	15.4	15.7
Ala	13.6	12.1	14.3	10.5	8.5	9.8	9.8	13.8	13.3	11.1	10.1	9.9	10.0	11.1	10.3	10.3	10.1	9.9	10.3	10.0
Val	4.2	1.7	3.4	2.3	2.3	2.2	2.5	3.4	3.8	3.0	2.8	3.3	3.5	3.5	3.4	3.8	3.8	3.1	3.8	3.6
Cys	0.4	–	–	0.4	–	–	–	0.1	–	–	0.8	0.4	0.4	–	0.3	0.9	0.9	0.4	0.7	0.6
Met	0.7	0.5	1.7	0.9	2.1	0.4	1.4	2.1	1.3	1.5	0.6	0.8	0.9	0.7	1.1	0.9	1.1	0.7	1.2	0.9
Ile	0.5	1.1	2.0	2.3	2.0	2.3	2.0	2.8	2.4	2.5	2.1	2.4	2.4	2.6	2.4	2.6	2.6	2.4	2.5	2.6
Leu	12.1	4.1	9.2	8.0	6.8	8.6	6.6	9.6	9.4	7.5	7.8	8.6	9.3	8.9	8.4	8.8	8.6	8.5	8.4	8.7
Tyr	1.7	1.0	2.4	1.4	2.3	3.1	1.4	3.0	1.8	1.9	4.1	3.9	4.2	4.0	3.9	4.6	4.4	4.4	4.4.	4.0
Phe	4.9	1.7	3.5	3.0	3.0	3.0	2.7	3.2	3.9	3.0	2.2	2.5	2.5	2.4	2.6	2.5	2.6	2.5	2.7	2.7
Lys	7.9	3.9	7.1	6.7	4.0	5.4	5.5	5.1	5.5	8.4	8.2	5.7	5.9	4.3	5.6	5.6	6.1	6.1	5.7	6.8
His	5.4	1.0	1.4	1.5	2.3	1.9	2.3	1.1	1.3	2.0	2.1	1.6	1.4	1.5	1.6	1.7	1.7	1.5	1.7	1.6
Trp	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Arg	2.9	4.5	4.2	3.9	9.1	7.7	2.9	4.4	3.7	3.2	4.4	4.5	4.6	4.4	4.3	4.6	4.6	4.8	4.5	4.5
Amount [μg/mg]	622	337	313	102	281	261	95	395	86	194	279	390	244	273	202	359	406	283	358	423

feeding groups (corresponding to animal, bacterial, fungal and herbal origin of the food), all classifications errors as well as the out-of-bag error (OOB error) rates were 0%. Hence, all animals were 100% correctly assigned based on their fatty acid profiles.

3.2. Amino acid composition of resources and mites

The amino acid composition of the ten resources and the according mites differed significantly (PERMANOVA: pseudo$F_{1,18} = 3.5$, $R^2 = 0.16$, $p = 0.002$; Table 2; Fig. 1C). Overall the composition of amino acids in mites was similar (mean distance = 0.02; Fig. 1C and D), while the composition in resources was more dispersed (mean distance = 0.12; Fig. 1C and D) and differed significantly (PERMDISP: $F_{1,18} = 15.9$, $p < 0.001$). The resources blood as well as bone were most separated from the others (Fig. 1C) and characterized by high ratios of Leu (12.1%) and His (5.4%) in blood as well as a high proportion of Gly (27.4%) and the exclusive amino acid OH-Pro (8.4%) in bone (Table 2). While chlorella, spirulina, wheat and yeast had a relatively similar composition (Table 2), fungi, hemp and lupine were characterized by high ratios of Glu (17.5–20.8%) with hemp being further distinguished via its high Arg (9.1%) content (Table 2). Pollen contained the highest percentage of Pro (24.8%). The amino acid composition of mites, however, was compared to the resources, highly homogeneous (Fig. 1D), only bone feeding mites exclusively contained OH-Pro (Table 2).

3.3. Dietary transfer of fatty and amino acids

The total amount of fatty acids was highly variable in the resources (Fig. 2A, Table 1) showing 55 ± 52 μg mg^{-1} and an according variation of 95%. To a lesser extend is was also the situation in mites (Table 1) showing a total fatty acid amount of 57 ± 27 μg mg^{-1} corresponding to a variation of 47%. The total amounts of fatty acids in the mites were highly correlated with the amounts in resources (Pearson's $r = 0.73$, $p < 0.016$; Fig. 2A). Furthermore, amounts of some fatty acids in resources/mites were highly correlated (C22:0, C24:0, C18:1Δ9 and the C18:1Δ9/C18:3Δ9,12,15 mixture; Table 3), while others were not (C16:0, C18:0, C18:2Δ9,12; Table 3). For most mite groups, namely animals

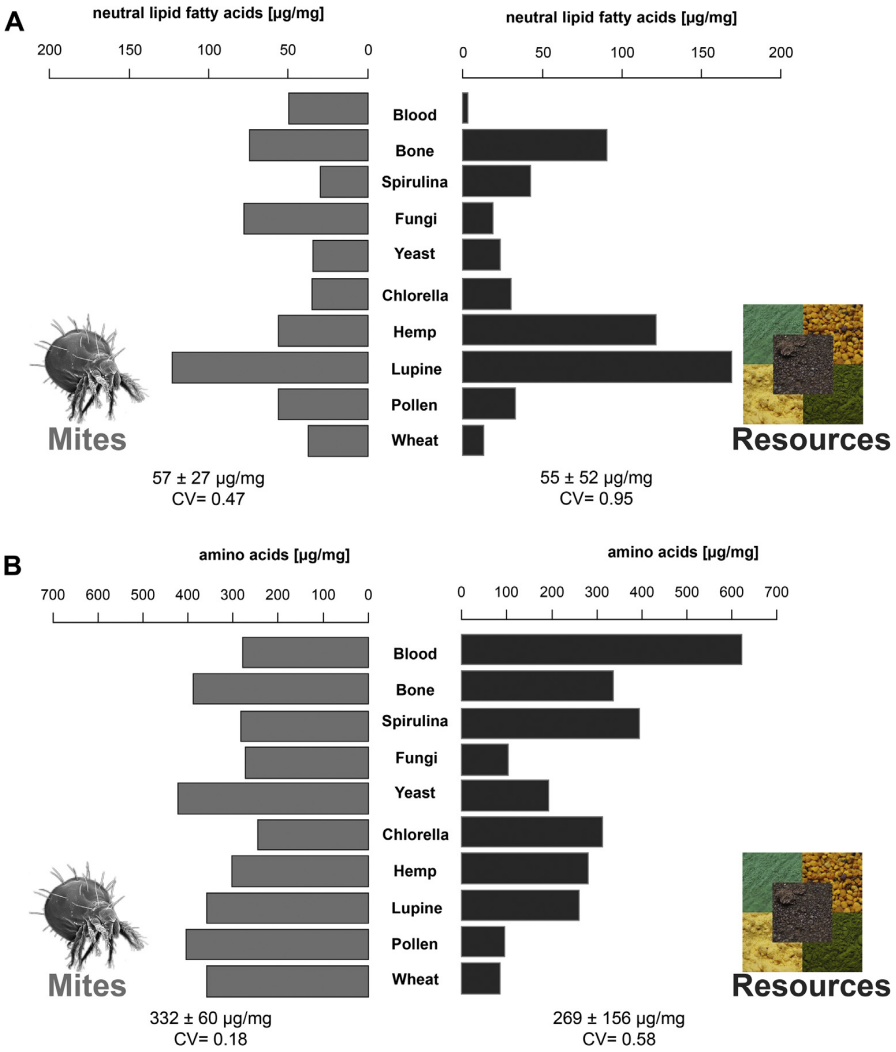


Fig. 2. Amounts [μg/mg] of neutral lipid fatty acids (A) and amino acids (B) of mites fed on the different resources (dark grey) and the corresponding resources (light grey).

Table 3
Correlation between the amount [μg/mg] of corresponding amino- and fatty acids of all resources and animals based on means.

	Pearson's r	p
Amino acids		
Ile	−0.30	0.39
Leu	−0.61	0.06
Lys	0.16	0.65
Met	−0.47	0.17
Phe	−0.58	0.08
Thr	−0.60	0.07
Tyr	−0.45	0.20
Val	−0.40	0.25
Fatty acids		
C16:0	0.27	0.44
C18:0	0.26	0.47
C22:0	0.99	<**0.001**
C24:0	0.91	<**0.001**
C16:1Δ9	0.89	<**0.001**
C18:1Δ9+ C18:3Δ9,12,15	0.85	**0.002**
C18:2Δ9,12	0.62	0.06

Significant p-values after false discovery rate correction are in bold.

feeding on blood, chlorella, fungi, pollen, wheat and yeast, the fatty acid amount increased, while it decreased in the other resources (bone, hemp, lupine and spirulina; Table 1).

The overall amount of amino acids in resources was 269 ± 156 μg mg^{-1}, thus showing a variation of 58%, compared to an overall amount of 332 ± 60 μg mg^{-1} and a variation of 18% in mites (Fig. 2B, Table 2). Neither the amount of all amino acids in resources and mites (Pearson's $r = -0.43$, $p = 0.22$; Fig. 2B), nor the amounts of different essential amino acids (see Pearson's r tests in Table 3) were correlated. In most mites amino acid amounts increased compared to resources (bone, fungi, hemp, lupine, pollen, wheat and yeast), however, in some resources they decreased (blood, chlorella and spirulina; Table 2, Fig. 2B).

4. Discussion

4.1. Biomarker functions and nutritional stoichiometry of fatty acids

The analysis of fatty acid patters has long been used to access information on microbial communities in soils and was more recently introduced to study soil food web links (Ruess and Chamberlain, 2010; Frostegård et al., 2011; and references therein). We here present the first controlled laboratory study investigating the applicability of fatty acids as biomarkers and tool to study trophic transfer in oribatid mites. Our results evidently show that the fatty acid composition of neutral lipids in the model mite species *A. longisetosus* is strongly influenced by its diet, hence conforming a direct incorporation of dietary fatty acids in oribatid mites ("dietary routing"). This is a common process in invertebrates which can assimilate fatty acids directly from their diet or biosynthesize them *de novo* (Canavoso et al., 2001). Usually, the direct incorporation of dietary lipids is favored since this is energetically beneficial (e.g. Stanley-Samuelson et al., 1988; Brandstetter and Ruther, 2016). Especially the strong correlation of fatty acid amounts in resources and mites confirm this mechanism for Oribatida and suggest a direct integration of these biomolecules into the fat body. However, the compositional differences of resources and mites are not surprising. Some fatty acids like C16:0 or C18:0 are typical constitutes of animal body fats (Rustan and Drevon, 2005) and hence can be synthesized *de novo* from sugars (Morgan, 2010; Shimizu et al., 2014). Therefore, these fatty acids are present in higher ratios in animals than in resources and may also explain the slightly higher compositional homogeneity of mites. Other fatty acids, like saturated > C20:0, or C16:1Δ9, C18:1Δ9 and C18:3Δ9,12,15 which are known as biomarkers (e.g. Ruess et al., 2007; Sechi et al., 2014) were highly correlated, confirming their function as trophic markers in oribatid mites. However, considering the general composition of the resources and mites, it is surprising that we found bacterial biomarkers in bone powder/bone mites, but no specific bacterial fatty acids in spirulina/spirulina mites. Furthermore, the ratio of C18:1Δ9 to C18:2Δ9,12 which was used to characterize between plant (higher ratios) or fungal (lower ratios) based diets before (Ruess et al., 2007; Pollierer et al., 2010) showed similar results to our data. In addition to the relative biomarker function for fungal/plant derived diet, the C18:1Δ9 to C18:2Δ9,12 ratio may also be used to detect animal-based food sources, since many terrestrial animals seem to contain high proportions of C18:1Δ9 (e.g. ants; see Rosumek et al., 2017). Since the spirulina powder we used surprisingly contained no specific bacterial NLFAs, also the C18:1Δ9 to C18:2Δ9,12 ratio may be misleading.

We provide strong experimental evidence, that NLFA patterns in oribatid mites can be used to unequivocally assign feeding guilds and possibly also to elucidate food-web/trophic connections of these animals. We also suggest that fatty acid patters could potentially, be used to relate field collected mites to food sources, which may relate to soil energy channels. Mites in this study were kept under *ad libitum* feeding conditions on one out of ten different resources. This approach is considered strong to elucidate strictly trophic transfer and dietary routing of biomolecules (Raubenheimer and Simpson, 1997), yet more experiments, especially about the effects of dietary switching are necessary, to interpret fatty acid patters of oribatid mites from the field, since they have been termed as "choosy generalists" (*sensu* Schneider and Maraun, 2005; see also Norton, 2007).

4.2. Biomarker functions and nutritional stoichiometry of amino acids

Information about amino acid compositions of soil organisms are limited to some studies of meso- and macroinvertebrates like pill millipedes or earthworms (e.g. Pokarzhevskii et al., 1997; Rawlins et al., 2006). Here, we present the first data on soil microinvertebrates, namely oribatid mites. Amino acid profiles of *A. longisetosus* are homeostatic and independent from dietary compositions. This finding supports studies of earthworms and pill millipedes, where amino acid composition was not associated or altered with soil nitrogen or different litter material (Rawlins et al., 2006; Xiang et al., 2006). Since there are no other storage molecules of amino acids than proteins (Limin et al., 2006), our results suggest a selective assimilation and compositional homeostasis for amino acids in oribatid mites, as it was observed for other invertebrate taxa (Anderson et al., 2004; Rawlins et al., 2006). Furthermore, the lack of correlation of the overall amount of amino acids and essential amino acids (as defined by Nation 2002) to the amino acid amounts in mites provide further evidence for selective resorption of distinct amounts of specific amino acids from resource proteins. Stability of the amino acid composition in *A. longisetosus* is also in accordance with Heethoff and Scheu (2016), who found a constant trophic enrichment of nitrogen of $\Delta^{15}N = 3.9‰$ in this species, irrespective of diet. Hence, the consistent amounts and stable compositional patterns of amino acids in oribatid mites confirm the hypothesis of Anderson et al. (2004) that most animals should possess a homeostasis of body protein. Our results illustrate that patterns of amino acids are unsuitable as nutritional biomarkers. However, the highly homeostatic properties of amino acids further underpin the reliability of ^{15}N as trophic level discriminator in oribatid mites. Yet, more laboratory and field studies are necessary

to monitor and quantify amino acid flow in soil energy channels, as well as their incorporation from above and below ground resources into the soil fauna.

Acknowledgments

Adrian Brückner was supported by a PhD scholarship from the German National Academic Foundation. This study was funded by the German Research Foundation (DFG; HE 4593/5-1).

References

Anderson, J.M., 1975. The enigma of soil animal diversity. In: Vanek, J. (Ed.), Proceedings of the 5th International Colloquium on Soil Zoology Held in Prague September 17-22, 1973. Academia, Prague, pp. 51–58.
Anderson, T.R., Boersma, M., Raubenheimer, D., 2004. Stoichiometry: linking elements to biochemicals. Ecology 85, 1193–1202.
Arthur, K.E., Kelez, S., Larsen, T., Choy, C.A., Popp, B.N., 2014. Tracing the biosynthetic source of essential amino acids in marine turtles using delta13C fingerprints. Ecology 95, 1285–1293.
Boschker, H.T.S., Middelburg, J.J., 2002. Stable isotopes and biomarkers in microbial ecology. FEMS Microbiology Ecology 40 (2), 85–95.
Brandstetter, B., Ruther, J., 2016. An insect with a delta-12 desaturase, the jewel wasp Nasonia vitripennis, benefits from nutritional supply with linoleic acid. Naturwissenschaften 103, 40.
Breiman, L., 2001. Random forest. Machine Learning 45, 5–32.
Brückner, A., Heethoff, M., 2017. A chemo-ecologists' practical guide to compositional data analysis. Chemoecology 27, 33–46.
Buse, T., Ruess, L., Filser, J., 2013. New trophic biomarkers for Collembola reared on algal diets. Pedobiologia 56, 153–159.
Butler, J., Tonn, R.J., 1963. Free amino acids in three species of mites. Annals of the Entomological Society of America 56, 406.
Canavoso, L.E., Jouni, Z.E., Karnas, K.J., Pennington, J.E., Wells, M.A., 2001. Fat metabolism in insects. Annual Review of Nutrition 21, 23–46.
Chamberlain, P.M., Bull, I.D., Black, H.I., Ineson, P., Evershed, R.P., 2004. Lipid content and carbon assimilation in Collembola: implications for the use of compound-specific carbon isotope analysis in animal dietary studies. Oecologia 139, 325–335.
Chamberlain, P.M., Bull, I.D., Black, H.I., Ineson, P., Evershed, R.P., 2005. Fatty acid compositions of Collembola fed differing diets: identification of trophic biomarkers. Soil Biology & Biochemistry 37, 1608–1624.
Dunkelblum, E., Tan, S.H., Silk, P.J., 1985. Double-bond location in monounsaturated fatty acids by dimethyl disulfide derivatization and mass spectrometry: application to analysis of fatty acids in pheromone glands of four lepidoptera. Journal of Chemical Ecology 11, 265–277.
Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226, 497–509.
Frostegård, A., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. Journal of Microbiological Methods 14, 151–163.
Frostegård, A., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biology & Biochemistry 43, 1621–1625.
Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4, 9.
Haubert, D., Häggblom, M.M., Langel, R., Scheu, S., Ruess, L., 2006. Trophic shift of stable isotopes and fatty acids in Collembola on bacterial diets. Soil Biology & Biochemistry 38, 2004–2007.
Heethoff, M., Scheu, S., 2016. Reliability of isotopic fractionation (Δ15N, Δ13C) for the delimitation of trophic levels of oribatid mites: diet strongly affects Δ13C but not Δ15N. Soil Biology & Biochemistry 101, 124–129.
Heethoff, M., Laumann, M., Bergmann, P., 2007. Adding to the reproductive biology of the parthenogenetic oribatid mite, Archegozetes longisetosus (Acari, Oribatida, Trhypochthoniidae). Turkish Journal of Zoology 31, 151–159.
Heethoff, M., Bergmann, P., Laumann, M., Norton, R.A., 2013. The 20th anniversary of a model mite: a review of current knowledge about Archegozetes longisetosus (Acari, Oribatida). Acarologia 53, 353–368.
Heidemann, K., Scheu, S., Ruess, L., Maraun, M., 2011. Molecular detection of nematode predation and scavenging in oribatid mites: laboratory and field experiments. Soil Biology & Biochemistry 43, 229–236.
Jones, D.L., Kielland, K., 2002. Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soils. Soil Biology & Biochemistry 34, 209–219.
Jones, D.L., Owen, A.G., Farrar, J.F., 2002. Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. Soil Biology & Biochemistry 34, 1893–1902.
Juen, A., Traugott, M., 2006. Amplification facilitators and multiplex PCR: tools to overcome PCR-inhibition in DNA-gut-content analysis of soil-living invertebrates. Soil Biology & Biochemistry 38, 1872–1879.
Leonhardt, S.D., Blüthgen, N., 2012. The same, but different: pollen foraging in honeybee and bumblebee colonies. Apidologie 43, 449–464.
Limin, L., Feng, X., Jing, H., 2006. Amino acids composition difference and nutritive evaluation of the muscle of five species of marine fish, Pseudosciaena crocea (large yellow croaker), Lateolabrax japonicus (common sea perch), Pagrosomus major (red seabream), Seriola dumerili (Dumeril's amberjack) and Hapalogenys nitens (black grunt) from Xiamen Bay of China. Aquaculture Nutrition 12, 53–59.
Maaß, S., Caruso, T., Rillig, M.C., 2015. Functional role of microarthropods in soil aggregation. Pedobiologia 58, 59–63.
Maraun, M., Erdmann, G., Fischer, B.M., Pollierer, M.M., Norton, R.A., Schneider, K., Scheu, S., 2011. Stable isotopes revisited: their use and limits for oribatid mite trophic ecology. Soil Biology & Biochemistry 43, 877–882.
Morgan, E.D., 2010. Biosynthesis in Insects. RSC Publishing, Camebridge.
Norton, R.A., 2007. Holistic acarology and ultimate causes: examples from the oribatid mites. In: Morales-Malacara, J.B., Behan-Pelletier, V., Ueckermann, E., Perez, T.M., Estrada-Venegas, E.G., Badii, M. (Eds.), Acarology XI: Proceedings of the International Congress. Sociedad Latinoamericana de Acarologia, Mexico, pp. 3–20.
Oksanen, J., Blachet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Wagner, H., 2016. Vegan: Community Ecology Package. R Package, version 2.3-4. http://CRAN.R-project.org/package=vegan.
Pokarzhevskii, A.D., Zaboyev, D.P., Ganin, G.N., Gordienko, S.A., 1997. Amino acids in earthworms: are earthworms ecosystemivorous? Soil Biology & Biochemistry 29, 559–567.
Pollierer, M.M., Langel, R., Scheu, S., Maraun, M., 2009. Compartmentalization of the soil animal food web as indicated by dual analysis of stable isotope ratios (15N/14N and 13C/12C). Soil Biology & Biochemistry 41, 1221–1226.
Pollierer, M.M., Scheu, S., Haubert, D., 2010. Taking it to the next level: trophic transfer of marker fatty acids from basal resource to predators. Soil Biology & Biochemistry 42, 919–925.
Pollierer, M.M., Dychmans, J., Scheu, S., Haubert, D., 2012. Carbon flux through fungi and bacteria into the forest soil animal food web as indicated by compound-specific 13C fatty acid analysis. Functional Ecology 26, 978–990.
R Core Team, 2015. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
Raubenheimer, D., Simpson, S.J., 1997. Integrative models of nutrient balancing: application to insects and vertebrates. Nutrition Research Reviews 10, 151–179.
Rawlins, A.J., Bull, I.D., Poirier, N., Ineson, P., Evershed, R.P., 2006. The biochemical transformation of oak (Quercus robur) leaf litter consumed by the pill millipede (Glomeris marginata). Soil Biology & Biochemistry 38, 1063–1076.
Remén, C., Krüger, M., Cassel-Lundhagen, A., 2010. Successful analysis of gut contents in fungal-feeding oribatid mites by combining body-surface washing and PCR. Soil Biology & Biochemistry 42, 1952–1957.
Rosumek, F.B., Brückner, A., Blüthgen, N., Menzel, F., Heethoff, M., 2017. Patterns and dynamics of neutral lipid fatty acids in ants – implications for ecological studies. Frontiers in Zoology. http://dx.doi.org/10.1186/s12983-017-0221-1.
Ruess, L., Chamberlain, P.M., 2010. The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biology & Biochemistry 42, 1898–1910.
Ruess, L., Häggblom, M.M., Zapata, E.J.G., Digton, J., 2002. Fatty acids of fungi and nematodes-possible biomarkers in the soil food chain? Soil Biology & Biochemistry 34, 745–756.
Ruess, L., Tiunov, A.V., Haubert, D., Richnow, H.H., Häggblom, M.M., Scheu, S., 2005. Carbon stable isotope fractionation and trophic transfer of fatty acids in fungal based soil food chains. Soil Biology & Biochemistry 37, 945–953.
Ruess, L., Schütz, K., Migge-Kleian, S., Häggblom, M.M., Kandeler, E., Scheu, S., 2007. Lipid composition of Collembola and their food resources in deciduous forest stands—implications for feeding strategies. Soil Biology & Biochemistry 39, 1990–2000.
Rustan, A.C., Drevon, C.A., 2005. Fatty Acids: Structures and Properties. eLS, New Jersey.
Scheu, S., Falca, M., 2000. The soil food web of two beech forests (Fagus sylvatica) of contrasting humus type: stable isotope analysis of a macro- and a mesofauna-dominated community. Oecologia 123, 285–296.
Scheu, S., Setälä, H., 2002. Multitrophic interactions in decomposer food webs. In: Tscharntke, T., Hawkins, B. (Eds.), Multitrophic Level Interactions. Cambridge University Press, Cambridge, pp. 223–264.
Scheu, S., Ruess, L., Bonkowski, M., 2005. Interactions between micro-organisms and soil micro- and mesofauna. In: Buscot, F., Varma, A. (Eds.), Soil Biology, Volume 3-Microorganisms in Soils: Roles in Genesis and Functions. Springer, New York, pp. 253–275.
Schneider, K., Maraun, M., 2005. Feeding preferences among dark pigmented fungi (Dematiacea) indicate trophic niche differentiation of oribatid mites. Pedobiologia 49, 61–67.
Schneider, K., Migge, S., Norton, R.A., Scheu, S., Langel, R., Reineking, A., Maraun, M., 2004. Trophic niche differentiation in soil microarthropods (Oribatida, Acari): evidence from stable isotope ratios (15N/14N). Soil Biology & Biochemistry 36, 1769–1774.
Sechi, V., D'Annibale, A., Ambus, P., Sárossy, Z., Krogh, P.H., Eriksen, J., Holmstrup, M., 2014. Collembola feeding habits and niche specialization in agricultural grasslands of different composition. Soil Biology & Biochemistry 74, 31–38.
Shimizu, N., Naito, M., Mori, N., Kuwahara, Y., 2014. De novo biosynthesis of linoleic acid and its conversion to the hydrocarbon (Z,Z)-6,9-heptadecadiene in the astigmatid mite, Carpoglyphus lactis: incorporation experiments with 13C-labeled glucose. Insect Biochemistry and Molecular Biology 45, 51–57.

Soong, J.L., Nielsen, U.N., 2016. The role of microarthropods in emerging models of soil organic matter. Soil Biology & Biochemistry 102, 37–39.
Stanley-Samuelson, D.W., Jurenka, R.A., Cripps, C., Blomquist, G.J., Derenobales, M., 1988. Fatty acids in insects - composition, metabolism, and biological significance. Archives of Insect Biochemistry and Physiology 9, 1–33.
Stein, S.E., 2015. Mass spectra by NIST mass spec data center. In: Linstrom, P.J., Mallard, W.G. (Eds.), NIST Chemistry WebBook, NIST Standard Reference Database Number 69, vol. 20899. National Institute of Standards and Technology, Gaithersburg MD. http://webbook.nist.gov.
Wehner, K., Norton, R.A., Blüthgen, N., Heethoff, M., 2016. Specialization of oribatid mites to forest microhabitats—the enigmatic role of litter. Ecosphere 7, e01336.
Wolkovich, E.M., 2016. Reticulated channels in soil food webs. Soil Biology & Biochemistry 102, 18–21.
Xiang, C., Zhang, P., Pan, G., Qiu, D., Chu, Q., 2006. Changes in diversity, protein content, and amino acid composition of earthworms from a paddy soil under different long-term fertilizations in the Tai Lake Region, China. Acta Ecologica Sinica 26, 1667–1673.
Zelles, L., Bai, Q.Y., Beck, T., Beese, F., 1992. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biology & Biochemistry 24, 317–323.

10.3.2 Publication 6: ***De novo* biosynthesis of simple aromatic compounds by an arthropod (*Archegozetes longisetosus*)**

Adrian Brückner, Martin Kaltenpoth and Michael Heethoff

Submitted to: Proceedings of the National Academy of Sciences of the United States of America

Submitted: 12.03.2017

Authors contributions:

MH and AB designed the research; AB and MK performed the experiments, AB, MK and MH analyzed the data; AB, MK and MH wrote the paper. All authors discussed and approved the final manuscript.

Brückner Adrian, Kaltenpoth Martin, Heethoff Michael. © The authors 2018

De novo biosynthesis of simple aromatic compounds by an arthropod (*Archegozetes longisetosus*)

Short title: Aromatic biosynthesis in an arthropod

Adrian Brückner[a,1], Martin Kaltenpoth[b], Michael Heethoff[a,1]

[a]Technische Universität Darmstadt, Ecological Networks, Schnittspahnstraße 3, 64287 Darmstadt, Germany

[b]Johannes Gutenberg University, Evolutionary Ecology, Institute of Organismic and Molecular Evolution, Johann-Joachim-Becher-Weg 13, 55128 Mainz, Germany

[1]Corresponding authors: heethoff@bio.tu-darmstadt.de, adrian.brueckner@gmail.com

ORCID:

Adrian Brückner: 0000-0002-9184-8562

Martin Kaltenpoth: 0000-0001-9450-0345

Michael Heethoff: 0000-0003-3453-4871

The authors declare no conflict of interest

Author contributions: M.H. and A.B. designed research; A.B., M.K. and M.H. performed research; A.B., M.K. and M.H. analyzed data; A.B. and M.H. wrote the paper.

Classification:

Major: Biological Sciences

Minor: Biochemistry, Physiology

Keywords:

Biosynthetic pathways; polyketides; chemical ecology; oribatid mites; chemical defense

Abstract (250)

The ability to synthesize simple aromatic compounds is well known from bacteria, fungi and plants, which all share an exclusive biosynthetic route – the shikimic acid pathway. Some of these organisms further evolved the polyketide pathway to form core benzenoids *via* a head-to-tail condensation of polyketide precursors. Arthropoda, the largest phylum of Metazoa, however, seem to lack the ability to synthesize aromatics and rely on aromatic amino acids acquired from food, or on symbiotic microorganisms that supplement them with benzenoid metabolites. Some studies on arthropod taxa (ants and harvestman), indicated that *de novo* biosynthesis of aromatics may occur *via* the polyketide pathway, however, without having excluded endosymbiotic bacteria these results remain inconclusive. We investigated the biosynthesis of aromatic compounds in defense secretions of the oribatid mite *Archegozetes longisetosus*. Exposing the mites to a diet containing high concentrations of antibiotics effectively removed microbial partners, as confirmed by quantitative PCR and fluorescence *in situ* hybridization, but did not affect the production of defensive benzenoids. To gain insights into the benzenoid biosynthetic pathway, we fed the mites with a variety of stable-isotope labeled precursor molecules (glucose, malonic acid, acetate, and the aromatic amino acid phenylalanine) and monitored incorporation of labelled atoms with mass spectrometry. While the phenylalanine benzene ring was not used directly, its breakdown products and those of glucose as well as malonic acid and acetate were incorporated into the benzenoid compounds of the defensive secretions. Our results hence indicate that the mites can autogenously synthesize simple benzenoids, likely *via* a polyketide-like pathway.

Significance statement (120)

Aromatic hydrocarbons and their derivatives are characterized by a cyclic six carbon-atom molecule with three conjugated double bounds – the benzene ring. Many aromatic hydrocarbons are used in chemical communication by bacteria, fungi, plants and animals.

While the autogenous biosynthesis of these compounds is well known for bacteria, fungi and plants – they have an exclusive biochemical pathway to produce the benzene rings as aromatic amino acids – most animals have been assumed to depend on a nutritional source or endosymbiotic bacteria for obtaining aromatic molecules. We show that a chelicerate arthropod, the oribatid mite *Archegozetes longisetosus*, autogenously synthesizes aromatic compounds *de novo*, without the aid of endosymbiotic microorganisms.

Introduction

Simple aromatic compounds (i.e. chemicals containing a benzene ring) are important products in chemical science and industry, but also in nature (1, 2). Overall, about 550 different simple and many more complex polyketide-derived aromatics have been described from bacteria, fungi, plants and animals (3, 4). The unique electronic structure of the benzene ring – a delocalized π-electron system with a six-ringed carbon skeleton – renders aromatics key structural motifs shaping interactions on both molecular and organismal levels (5, 6). In the primary metabolism of arthropods, aromatic amino acids are important building blocks of proteins, but also are utilized in cuticle formation, sclerotization and melanization (7, 8). Among secondary metabolites, arthropods use simple benzenoids in pheromonal communication and as defensive compounds, while the more complex polyketide aromatics also function as potent antibiotics (3, 9, 10).

Bacteria, fungi and plants evolved two biosynthetic routes to form benzene rings: the shikimic acid (1, 10) and the polyketide pathway (3) in which benzene rings arise *via* a head-to-tail condensation of poly-β-carbonyl intermediates followed by an intramolecular condensation forming the aromatic system (11, 12). While known from some lower animals and maybe vertebrates (3, 13), the ability of arthropods to synthesize simple aromatics *via* the polyketide pathway remains uncertain (10, 14). Aromatics are important for chemical interactions in insects, arachnids and myriapods as well (9), yet it is still unclear whether these

78 benzenoids are acquired from diet, synthesized *de novo* by the animals or by endosymbiotic
79 bacteria (3, 10, 14). It appears that especially the complex, large ringed polyketide aromatics
80 are mostly produced by bacteria (15-19), while simple aromatics may originate from a
81 potential arthropod polyketide pathway (20-23). Pankewitz & Hilker (14) reviewed available
82 studies on polyketides and concluded that there is no unequivocal evidence for *de novo*
83 biosynthesis of aromatics in arthropods, since potential endosymbiont contributions were not
84 excluded, and this still holds true (10, 24, 25). Only the study of Bestmann et al. (20) on
85 aromatic trail pheromones of formicine ants indicated that bacteria may not be involved in the
86 biosynthesis of these chemicals.
87 The oribatid mite *Archegozetes longisetosus* Aoki produces defensive secretions
88 containing two simple aromatics, 2-hydroxy-6-methyl-benzaldehyde (2,6-HMBD) and 3-
89 hydroxybenzene-1,2-dicarbaldehyde (γ-acaridial), in a pair of opisthonotal exocrine glands
90 (26). We reared animals in a controlled, sterile environment to investigate the ability to
91 synthesize these benzene-ringed compounds *de novo*. We performed labeling experiments
92 with different precursors under intensive antibiotic treatment to maintain symbiont-free
93 animals and to uncover the biosynthetic pathway of these aromatics. We demonstrate that 2,6-
94 HMBD and γ-acaridial are both synthesized *de novo*, most likely from poly-β-carbonyl (e.g.,
95 acetyl-CoA and malonyl-CoA).
96
97 **Results and Discussion**
98 The defensive chemicals of the oribatid mite *A. longisetosus* consist of a blend of ten
99 compounds (Fig. 1A) including two terpenes (~ 45 %), six hydrocarbons (~ 15 %) and two
100 aromatic compounds (~ 40 %) (26, 27). While the hydrocarbons probably serve as solvents,
101 the terpenes and aromatics are bioactive compounds used as alarm pheromones and predator
102 repellants (28).

In a first feeding experiment we tested if elimination of potentially symbiotic gut bacteria influences the biosynthesis of 2,6-HMBD and γ-acaridial by feeding the mites with food containing 10% antibiotic drugs (amoxicillin, streptomycin and tetracycline). We found that production of 2,6-HMBD and γ-acaridial was unaffected by treatments with individual antibiotics or with all three in concert (Fig. 1B; U-test: z= -1.3, p= 0.19; n= 32, Fig. S1). The combined treatment, which was used in further incorporation experiments with labeled precursors, effectively eliminated bacteria in the mites (Fig. 1C; U-test: z= -2.1, p= 0.027; n= 13). These results were also supported by fluorescence *in situ* hybridization (FISH), revealing that all detectable bacteria found on the food and in the alimentary tract (Fig. 1D) were eliminated in the antibiotic-treated mites (Fig. 1E). We detected no bacteria in the glands (Fig. 1F) or the digestive caecae (Fig. 1G), irrespective of antibiotic treatment, further supporting the general absence of endosymbiotic bacteria. By contrast, bacteria on the outer cuticle of the mites remained unaffected by antibiotic treatment and served as an internal control for FISH (Fig. S2). Hence, the gland does not house bacteria involved in the production of defensive compounds or precursors, as described for defensive symbioses in other arthropods (15, 17, 29, 30). Also the caecae, which are directly connected with the glandular tissue *via* a plasma mass (31), appear to be no brood chambers for bacteria.

In a second experiment, the diet of *A. longisetosus* was supplemented with food containing 25% of a stable isotope-labeled precursor - [$^{13}C_6$, d_7] D-glucose, [$^{13}C_3$] malonic acid, sodium [$^{13}C_1$] acetate or [$^{13}C_6$] phenylalanine - and 10% antibiotics. To examine the incorporation and possible biosynthesis of 2,6-HMBD (Fig. 2) and γ-acaridial we compared selected fragment ions using mass spectrometry and calculated an enrichment factor based on relative intensities. Both aromatics showed consistently enriched $[M+1]^+$ to $[M+8]^+$-ion series for [$^{13}C_6$, d_7] D-glucose, [$^{13}C_3$] malonic acid and sodium [$^{13}C_1$] acetate. Enrichment was strongest for the most general source, the heavily labeled D-glucose, while the more specific β-carbonyl precursors showed less enrichment (Tab. 1) for both compounds (see Fig. S3 for

2,6-HMBD and Fig. S4 for γ-acaridial). When feeding [$^{13}C_6$] phenylalanine, we found no enrichment of the $[M+6]^+$-ion (Tab. 1, Figs. S3 & S4), which would be expected for the incorporation of the completely ^{13}C-labeled benzene ring (32). Instead, we again found a stepwise enrichment in the $[M+1]^+$ to $[M+8]^+$-ion series (Tab. 1, Figs. S3 & S4), indicating that only fragments of the benzene ring of [$^{13}C_6$] phenylalanine had been incorporated into 2,6-HMBD and γ-acaridial after the mites had catabolized the amino acids to poly-β-carbonyl units (33, 34). Due to the elimination of bacteria, this strongly supports a *de novo* biosynthesis of both aromatic compounds.

All experimental data point to a head-to-tail condensation of poly-β-carbonyls and a subsequent cyclization of a C8-polyketide intermediate to the final aromatic compound (Fig. 3). This biosynthetic scenario fits well with the few other studies on benzenoid ant pheromones (20, 22, 23) and benzoquinoid defensive chemicals of harvestmen (21) that indicated a polyketide origin of these compounds, but did not exclude or specifically test for an involvement of potentially symbiotic microorganisms (35-37). Among more closely related arachnids, the storage mite *Chortoglyphus arcuatus* Troupeau produces an aliphatic polyketide-derived aggregation pheromone – (4*R*,6*R*,8*R*)-4,6,8-trimethyldecan-2-one – synthesized from one acetate and four propionates (38), further supporting the ability of mites to produce polyketides *de novo*.

In summary, the oribatid mite *A. longisetosus* synthesizes its simple aromatic defense compounds *de novo*, probably *via* the polyketide pathway (Fig. 3). We suspect this to be indicative of a more general ability of arthropods to biosynthesize many of their simple benzenoid molecules themselves (21-23). The enzymatic machinery and genes coding for these reactions remain to be elucidated. While they may not directly depend on bacterial symbionts (20), it remains possible that the polyketide synthase was acquired by horizontal gene transfer from previously symbiotic microorganisms (14, 39).

155 **Materials and methods**

156 **Mites**. The lineage 'ran' (40) of the pantropical, parthenogenetic oribatid mite
157 *Archegozetes longisetosus* was used in this study. Experimental cultures were established
158 from an already existing line feeding on wheat grass, *Triticum* sp. powder from Naturya
159 (Bath, UK) as follows: first, we collected eggs of *A. longisetosus* and surface-washed them
160 with sodium hypochlorite solution (3% w/v), ethanol (70% v/v) and sterilized water for 5 sec,
161 15 sec and 30 sec, respectively. Afterwards, eggs were transferred to sterile Petri dishes (ø 45
162 mm) lined with 1 cm sterilized plaster of Paris. Sterile cultures were maintained in a laminar-
163 flow closet at 28°C and 90% relative humidity. Sterilized water and 3-5 mg wheat grass or
164 different food mixtures (see below) were provided three times each week.

165 **Antibiotics feeding experiment.** In the first experiment we fed four different mixtures
166 of antibiotic-laden wheat grass (10% antibiotics, w/w) and pure wheat grass as a control to
167 different groups of 150 mites for one generation (approx. 50 days). We prepared 10% (w/w)
168 mixtures of sterilized wheat grass powder with amoxicillin, streptomycin and tetracycline as
169 well as a mixture of all three antibiotics (3.3% w/w for each). One week after the adult
170 eclosion, the defensive glands were extracted in hexane and chemically analyzed (see below).

171 **Feeding experiments with labeled precursors.** For the second experiment we used
172 only the 10% (w/w) mixture which contained all three antibiotics. Additionally, we added
173 25% (w/w) stable isotope-labeled precursors. We prepared four different mixtures with [$^{13}C_6$,
174 d_7] D-glucose, [$^{13}C_3$] malonic acid, sodium [$^{13}C_1$] acetate and [$^{13}C_6$] phenylalanine (all > 99%
175 enrichment, Sigma-Aldrich, St. Louis, USA) as well as a control with untreated wheat grass.
176 Again, cultures were maintained for one generation and glands of adult specimens were
177 extracted one week after eclosion using hexane (see below).

178 **Fluorescence *in situ* hybridization (FISH)**. For the control as well as the [$^{13}C_6$, d_7]
179 D-glucose and [$^{13}C_6$] phenylalanine treatments (both with 10% antibiotics, see above) three

180 entire specimens of *A. longisetosus* were fixated in 4% paraformaldehyde in PBS, and FISH
181 was performed on semi-thin sections as described previously (41, 42). The fixated samples
182 were dehydrated in a graded ethanol series and then embedded in cold-polymerizing resin
183 (Technovit 8100; Heraeus Kulzer, Hanau, Germany) according to manufacturer's instructions.
184 Sections of 7 µm thickness were obtained with a steel knife on a HM355S microtome (Leica,
185 Germany) and mounted on microscope slides coated with poly-L-lysine (Kindler, Freiburg,
186 Germany). FISH was done with the general eubacterial probes EUB388-Cy5 (5'-
187 GCTGCCTCCCGTAGGAGT-3') (43) and EUB784-Cy3 (5'-
188 TGGACTACCAGGGTATCTAATCC-3') (44) (two individuals each), or a combination of
189 EUB338-Cy3 and the general yeast probe PF2-Cy5 (5'-CTCTGGCTTCACCCTATTC-3')
190 (45) (one individual each). Samples were incubated for 90 minutes at 60°C in 100 µl
191 hybridization buffer (0.9M NaCl, 0.02M Tris/HCl pH 8.0, 0.01% SDS) containing 5 µl of
192 each probe (500 nM) as well as DAPI (4',6-diamidino-2-phenylindole) for counterstaining of
193 host cell nuclei. Two wash steps with pre-warmed washing buffer (0.1 M NaCl, 0.02 M
194 Tris/HCl pH8.0, 0.01% SDS, 5 mM EDTA), the second for 20 minutes at 60°C, as well as
195 rinsing with dH_2O served to remove residual probes. After drying at room temperature, slides
196 were covered with VectaShield and inspected on an AxioImager.Z1 fluorescence microscope
197 (Zeiss, Jena, Germany).
198 **Quantitative PCR**. To assess the effect of antibiotic treatment on absolute numbers of
199 bacteria associated with the mites, bacterial 16S rRNA copy numbers were determined by
200 quantitative PCR (qPCR). Since FISH experiments had revealed the presence of bacteria on
201 the surface, the mites were surface-washed in 5% (v/v) sodium dodecyl sulfate solution before
202 bacterial quantification. For the control and the [$^{13}C_6$, d_7] D-glucose + 10% antibiotics
203 treatment, DNA was extracted from eight replicates of 15-25 mites each, using the
204 MasterPure^{TM} DNA purification kit (Epicentre Technologies) according to manufacturer's
205 instructions. As a quality control of the DNA extracts and for later standardization of bacterial

206 titers, the DNA extracts were subjected to a qPCR with primers targeting the host 28S rRNA
207 gene (D3A _F: 5'-GACCCGTCTTGAAACACGGA-3'; and D3B_R: 5'-
208 TCGGAAGGAACCAGCTACTA-3') (46). Subsequently, samples that showed amplification
209 for the host 28S (five of the control replicates and all eight of the antibiotic treatment) were
210 subjected to a qPCR with general eubacterial 16S rRNA gene primers (Univ16SRT-F: 5'-
211 ACTCCTACGGGAGGCAGCAGT-3'; Univ16SRT-R: 5'-TATTACCGCGGCTGCTGGC-3')
212 (47). QPCRs were done on a RotorGene-Q cycler (Qiagen, Hilden, Germany) in final reaction
213 volumes of 25 µl, including the following components: 1 µl of DNA template, 2.5 µl of each
214 primer (10 µM), 6.5 µl of autoclaved distilled H_2O, and 12.5 µl of SYBR Green Mix (Qiagen,
215 Hilden, Germany). PCR conditions included 95°C for 5 minutes, followed by 40 cycles of
216 95°C for 10 seconds, 70°C for 15 seconds, and 72°C for 10 seconds. A melting curve analysis
217 was performed by increasing the temperature from 60 °C to 95 °C within 20 min. Standard
218 curves were established for the host 28S and bacterial 16S assays by using 10^3 to 10^{10} copies
219 of PCR product as templates. A Qubit fluorometer (Thermo Fisher Scientific) was used to
220 measure DNA concentrations for the templates of the standard curve. The ratio between
221 absolute copy numbers of bacterial 16S and host 28S (=bacterial/host copy ratio) was used as
222 a measure of bacterial abundance per sample.
223 **Chemical Analysis.** Gland exudates, containing the two studied aromatics, were
224 extracted from living mites by submersing individuals (antibiotics feeding experiment) or
225 groups of 15 (labeling experiment) in 50 µl hexane for 3 minutes. Crude hexane extracts (2-5
226 µl) were analyzed with a GCMS-QP2010 Ultra gas chromatography – mass spectrometry
227 (GCMS) system from Shimadzu (Kyōto, Japan) equipped with a ZB-5MS capillary column
228 (0.25 mm x 30m, 0.25 µm film thickness) from Phenomenex (Torrance, USA). Hydrogen was
229 used a carrier gas with a flow rate of 3.00 ml/min, with splitless injection and a temperature
230 ramp was set to increase from 50°C (5min) to 210°C at a rate of 6°C/min, followed by

35°C/min up to 320°C (for 5 min). Electron ionization mass spectra were recorded at 70 eV and characteristic fragment ions were monitored in single ion mode.

Data analysis. For the antibiotic feeding experiment we quantified the ion abundance and calculated the relative composition of aromatics (2,6-HMBD and γ-acaridial combined) compared to ion abundance of the other compounds on an individual base. Then we compared the (%) aromatic compounds among groups or treatments with a Kruskal-Wallis test or Mann-Whitney-U-tests, respectively. The bacterial/host copy ratio was analyzed with a Mann-Whitney-U-test as well. Statistics were performed in PAST 3.17 (48). For stable isotope enrichment, we compared the four treatment groups with the control and calculated the enrichment factors (EF) as $EF= (r_{treatment}-r_{control})/r_{control}$, where $r_{treatment}$ is the relative abundance (% relative to M^+) of a respective ion in the treatment GCMS analyses and $r_{control}$ is the relative intensity (%) of the same ion in the control group.

Acknowledgements

We thank Roy A. Norton for improving the manuscript and Benjamin Weiss, Dagmar Klebsch and Maximilian Maschler for their technical assistance. Adrian Brückner was supported by a PhD scholarship from the German National Academic Foundation. This study was funded by the German Research Foundation (DFG; HE 4593/5-1).

References

1. Walsh C & Tang Y (2017) *Natural product biosynthesis : chemical logic and enzymatic machinery* (RSC Publishing, Camebridge,UK).
2. Rocke AJ (2015) It began with a daydream: the 150th anniversary of the Kekule benzene structure. *Angew Chem Int Ed Engl* 54:46-50.
3. Hertweck C (2009) The biosynthetic logic of polyketide diversity. *Angew Chem Int Ed Engl* 48:4688-4716.
4. El-Sayed AM (2018) The Pherobase: database of pheromones and semiochemicals. (<http://www.pherobase.com>).
5. Meyer EA, Castellano RK, & Diederich F (2003) Interactions with aromatic rings in chemical and biological recognition. *Angew Chem Int Ed Engl* 42:1210-1250.
6. Riley KE & Hobza P (2013) On the importance and origin of aromatic interactions in chemistry and biodisciplines. *Acc Chem Res* 46:927-936.
7. Brunet P (1980) The metabolism of the aromatic amino acids concerned in the cross-linking of insect cuticle. *Insect Biochem* 10:467-500.
8. Chen P (1966) Amino acid and protein metabolism in insect development. *Adv In Insect Phys* 3:53-132.
9. Eisner T, Eisner M, & Siegler M (2005) *Secret Weapons* (Harvard Univ Press, Cambridge, MA) p 372.
10. Morgan ED (2010) *Biosynthesis in Insects* (RSC Publishing, Camebridge, UK).
11. Birch AJ & Donovan FW (1953) Studies in relation to biosynthesis. I. some possible routes to derivatives of orcinol and phloroglucinol. *Austr J Chem* 6:360-368.
12. Harris TM & Harris CM (1977) Synthesis of polyketide-type aromatic natural products by biogenetically modeled routes. *Tetrahedron* 33:2159-2185.

13. Castoe TA, Stephens T, Noonan BP, & Calestani C (2007) A novel group of type I polyketide synthases (PKS) in animals and the complex phylogenomics of PKSs. *Gene* 392:47-58.

14. Pankewitz F & Hilker M (2008) Polyketides in insects: ecological role of these widespread chemicals and evolutionary aspects of their biogenesis. *Biol Rev* 83:209-226.

15. Kellner RL (1999) What is the basis of pederin polymorphism in *Paederus riparius* rove beetles? The endosymbiotic hypothesis. *Entomol Exp Appl* 93:41-49.

16. Kellner RL (2001) Suppression of pederin biosynthesis through antibiotic elimination of endosymbionts in *Paederus sabaeus*. *J Insect Physiol* 47:475-483.

17. Piel J (2009) Metabolites from symbiotic bacteria. *Nat Prod Rep* 26:338-362.

18. Piel J, *et al.* (2005) Exploring the chemistry of uncultivated bacterial symbionts: antitumor polyketides of the pederin family. *J Nat Prod* 68(3):472-479.

19. Piel J, *et al.* (2004) Antitumor polyketide biosynthesis by an uncultivated bacterial symbiont of the marine sponge *Theonella swinhoei*. *Proc Natl Acad Sci USA* 101:16222-16227.

20. Bestmann HJ, Übler E, & Hölldobler B (1997) First biosynthetic studies on trail pheromones in ants. *Angew Chem Int Ed Engl* 36:395-397.

21. Rocha DF, *et al.* (2013) Harvestman phenols and benzoquinones: characterisation and biosynthetic pathway. *Molecules* 18:11429-11451.

22. Sun C-M & Toia RF (1993) Biosynthetic studies on ant metabolites of mellein and 2,4-dihydroxyacetophenone from $^{1,2\text{-}13}C_2$acetate. *J Nat Prod* 56:953-956.

23. Tecle B, Briophy JJ, & Toia RF (1986) Biosynthesis of 2-hydroxy-6-methylacetophenone in an Australian ponerine ant, *Rhytidoponera aciculata* (Smith). *Insect Biochem* 16:333-336.

298 24. Nazareth TM, Sudatti DB, & Machado G (2016) Chemical defense as a condition-
299 dependent trait in harvestmen. *J Chem Ecol* 42:1047-1051.
300 25. Raspotnig G, Schaider M, Föttinger P, & Schönhofer A (2017) A model for
301 phylogenetic chemosystematics: evolutionary history of quinones in the scent gland
302 secretions of harvestmen. *Front Ecol Evol* 5:139.
303 26. Sakata T & Norton RA (2003) Opisthonotal gland chemistry of a middle-derivative
304 oribatid mite, *Archegozetes longisetosus* (Acari: Trhypochthoniidae). *Int J Acarol*
305 29:345-350.
306 27. Brückner A & Heethoff M (2017) The ontogeny of oil gland chemistry in the oribatid
307 mite *Archegozetes longisetosus* Aoki (Oribatida, Trhypochthoniidae). *Int J Acarol*
308 43:337-342.
309 28. Raspotnig G (2006) Chemical alarm and defence in the oribatid mite *Collohmannia*
310 *gigantea* (Acari: Oribatida). *Exp Appl Acarol* 39:177-194.
311 29. Kaltenpoth M & Engl T (2014) Defensive microbial symbionts in hymenoptera. *Func*
312 *Ecol* 28:315-327.
313 30. Florez LV, Biedermann PHW, Engl T, & Kaltenpoth M (2015) Defensive symbioses
314 of animals with prokaryotic and eukaryotic microorganisms. *Nat Prod Rep* 32:904-
315 936.
316 31. Raspotnig G, Schuster R, & Krisper G (2003) Functional anatomy of oil glands in
317 *Collohmannia gigantea* (Acari, Oribatida). *Zoomorphology* 122:105-112.
318 32. Babst BA, Harding SA, & Tsai CJ (2010) Biosynthesis of phenolic glycosides from
319 phenylpropanoid and benzenoid precursors in populus. *J Chem Ecol* 36:286-297.
320 33. Bender DA (2012) *Amino acid metabolism* (John Wiley & Sons Ltd, Hoboken, NJ).
321 34. Mazelis M (1980) Amino acid catabolism. *Amino acids and derivatives - a*
322 *comprehensive treatise*, ed Miflin BJ (Acad Press, Cambridge, MA).

323 35. Feldhaar H (2011) Bacterial symbionts as mediators of ecologically important traits of
324 insect hosts. *Physiolo Entomol* 36:533-543.
325 36. Ferrari J & Vavre F (2011) Bacterial symbionts in insects or the story of communities
326 affecting communities. *Philos Trans R Soc Lond B Biol Sci* 366:1389-1400.
327 37. Gibson CM & Hunter MS (2010) Extraordinarily widespread and fantastically
328 complex: comparative biology of endosymbiotic bacterial and fungal mutualists of
329 insects. *Ecol Lett* 13(2):223-234.
330 38. Schulz S, Fuhlendorff J, Steidle JL, Collatz J, & Franz JT (2004) Identification and
331 biosynthesis of an aggregation pheromone of the storage mite *Chortoglyphus*
332 *arcuatus*. *Chembiochem* 5:1500-1507.
333 39. Ginolhac A, *et al.* (2005) Type I polyketide synthases may have evolved through
334 horizontal gene transfer. *J Mol Evol* 60:716-725.
335 40. Heethoff M, Bergmann P, Laumann M, & Norton RA (2013) The 20th anniversary of
336 a model mite: A review of current knowledge about *Archegozetes longisetosus* (Acari,
337 Oribatida). *Acarologia* 53:353-368.
338 41. Weiss B & Kaltenpoth M (2016) Bacteriome-localized intracellular symbionts in
339 pollen-feeding beetles of the genus *Dasytes* (Coleoptera, Dasytidae). *Front Microbiol*
340 7:1486.
341 42. Kaltenpoth M, Yildirim E, Gürbüz MF, Herzner G, & Strohm E (2012) Refining the
342 roots of the beewolf-*Streptomyces* symbiosis: antennal symbionts in the rare genus
343 *Philanthinus* (Hymenoptera, Crabronidae). *Appl Environ Microbiol* 78:822-827.
344 43. Amann RI, *et al.* (1990) Combination of 16S ribosomal RNA targeted oligonucleotide
345 probes with flow-cytometry for analyzing mixed microbial populations. *Appl Environ*
346 *Microbio.* 56:1919-1925.

347 44. Pakwan C, *et al.* (2017) Bacterial communities associated with the ectoparasitic mites
348 *Varroa destructor* and *Tropilaelaps mercedesae* of the honey bee (*Apis mellifera*).
349 *FEMS Microbiol Ecol* 93:10.1093/femsec/fix160.
350 45. Kempf VAJ, Trebesius K, & Autenrieth IB (2000) Fluorescent in situ hybridization
351 allows rapid identification of microorganisms in blood cultures. *J Clin Microbiol*
352 38:830-838.
353 46. Laumann M, *et al.* (2007) Speciation in the parthenogenetic oribatid mite genus
354 *Tectocepheus* (Acari, Oribatida) as indicated by molecular phylogeny. *Pedobiologia*
355 51:111-122.
356 47. Clifford RJ, *et al.* (2012) Detection of bacterial 16S rRNA and identification of four
357 clinically important bacteria by real-time PCR. *PLOS ONE* 7:e48558.
358 48. Hammer Ø, Harper DAT, & Ryan PD (2001) PAST: Paleontological statistics
359 software package for education and data analysis. *Palaeontol Electronica* 4:9.
360

Figure legends

Figure 1. Mites (*Archegozetes longisetosus*) contain two simple aromatic compounds in their defensive secretions, and reduction of bacteria did not affect their production. (A) Representative GC trace of gland extracts from *A. longisetosus*; in order of retention time: 2-hydroxy-6-methyl-benzaldehyde (2,6-HMBD), neral, neryl formate, tridecane, 3-hydroxybenzene-1,2-dicarbaldehyde (γ-acaridial). Pentadec-7-ene, petadecane, heptadeca-6,9-diene, heptadec-8-ene, heptadecane are not shown. (B) Supplementing a wheat grass diet ("control") with a mixture of antibiotics (10% w/w; combined amoxicillin, streptomycin and tetracycline; "antibiotics") did not affect the relative amount of aromatic compounds, but resulted in significantly lower bacterial load in *A. longisetosus* (C). Fluorescence *in situ* hybridization revealed a high bacterial prevalence in the food bolus of control group mites (D), while no detectable bacteria (E) were found in similar bolus in antibiotic-treated mites. Even in the control group, no bacteria were detected in the gland (F; the white arrow head marks the center of the gland) or in the caecae (i.e. pairwise sac-like organ that are located close to the glandular tissue; G). Bacteria stained in green with the general bacterial probe EUB338-Cy5; the green arrow heads mark bacterial signals detected in the food bolus (D) or on the cuticle (F). Colored bars represent means, error indicators are standard errors. The scale bar is 20 μm. ns= not significant: $p > 0.05$, *= significant: $p < 0.05$.

Figure 2. Representative mass spectra of unlabeled and ^{13}C/d-labeled 2-hydroxy-6-methyl-benzaldehyde recorded in single-ion mode. Mites fed with a mixture of labeled [$^{13}C_6$, d_7] D-glucose and antibiotics show enriched ions.

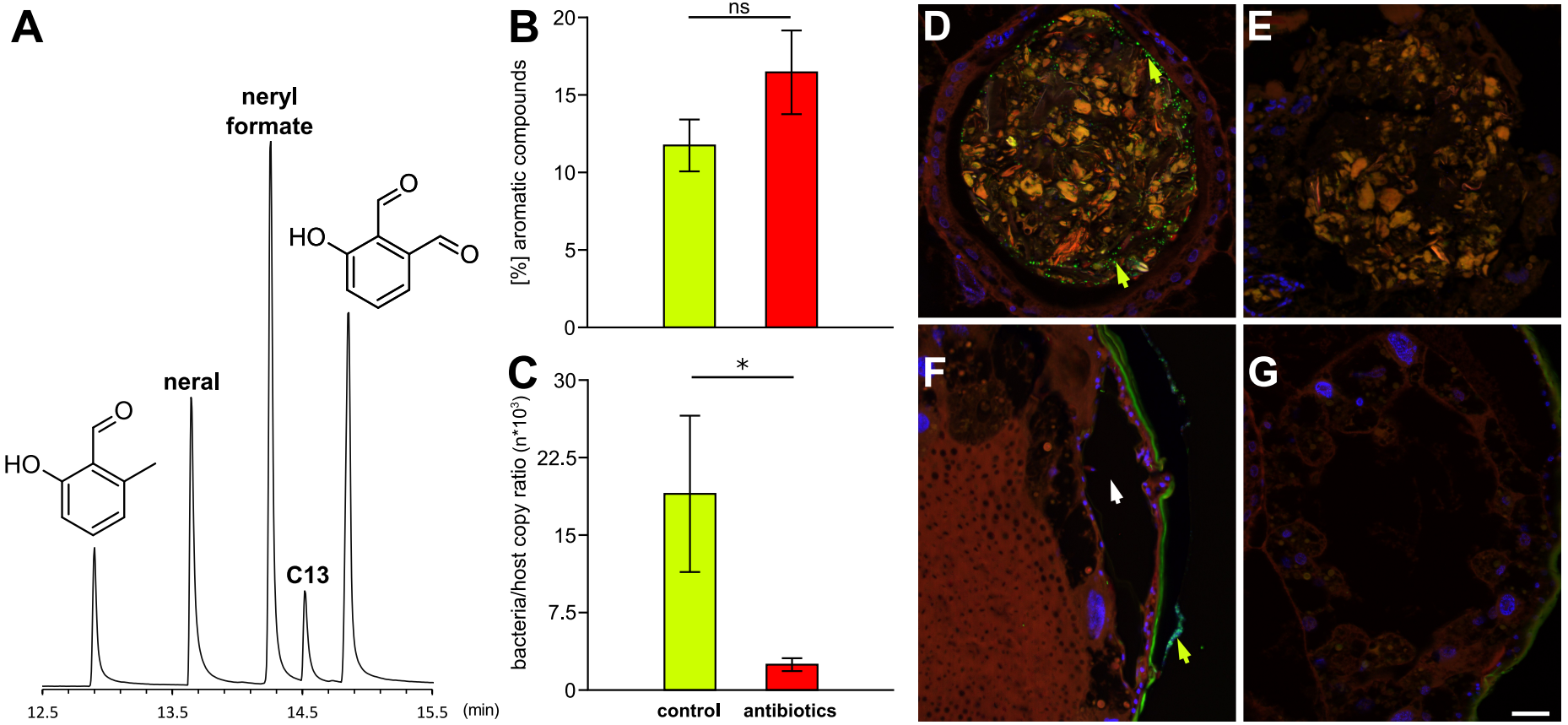
Figure 3. One proposed biosynthetic scenario leading to 2-hydroxy-6-methyl-benzaldehyde (2,6-HMBD) in the oribatid mite *Archegozetes longisetosus*. The mite uses (poly)-β-carbonyls like malonyl-CoA and/or acetyl-CoA to form a C8-polyketid intermediate, which finally does a ring closing cyclization yielding 2,6-HMBD. A direct integration of the fully ^{13}C-labeled benzene ring in phenylalanine was not observed. The second aromatic

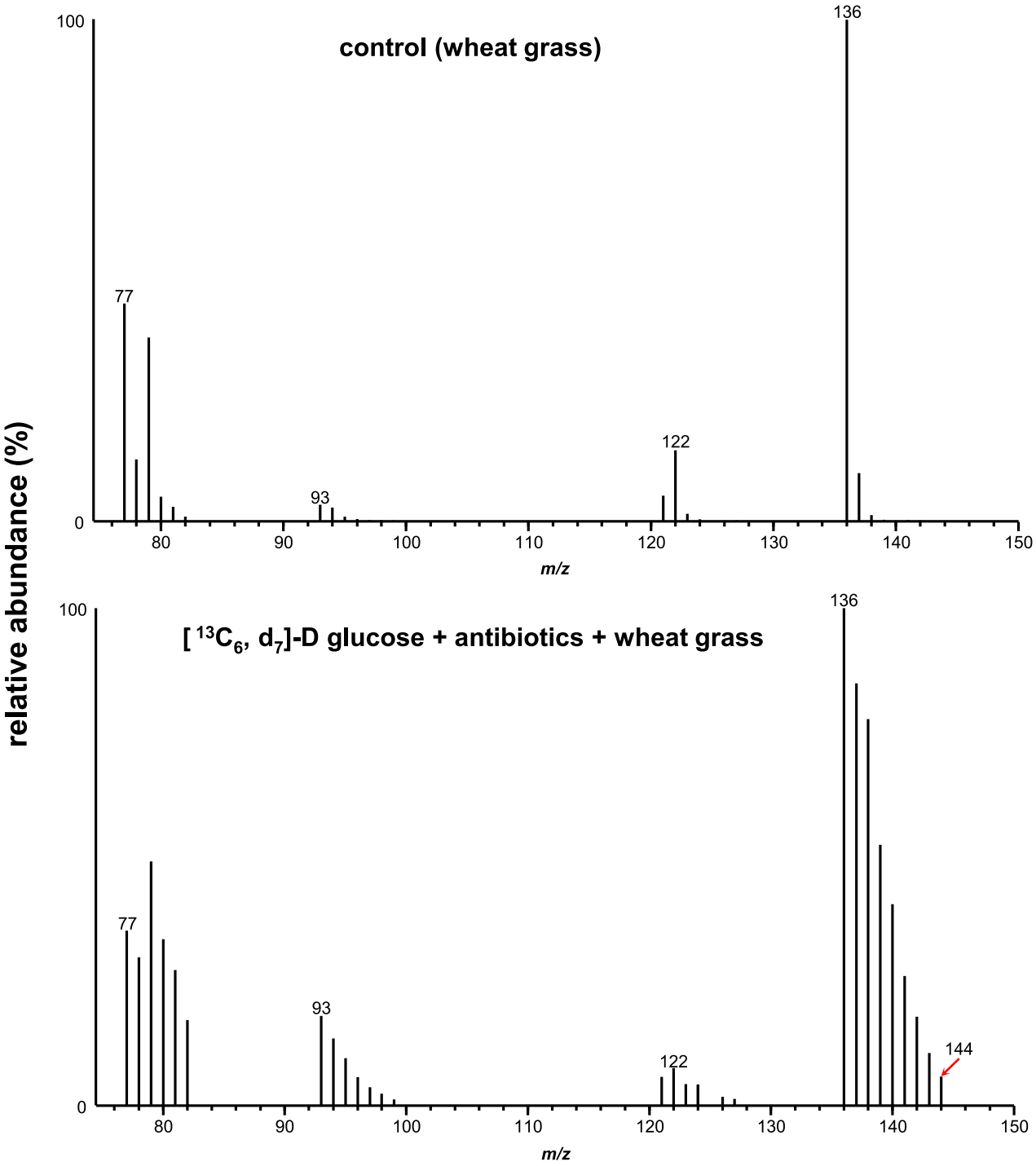
387 compound (3-hydroxybenzene-1,2-dicarbaldehyde; not shown) may be biosynthesized from
388 2,6-HMBD by an enzymatic oxidation of the methyl group to the corresponding aldehyde or
389 *via* a different head-to-tail condensation.

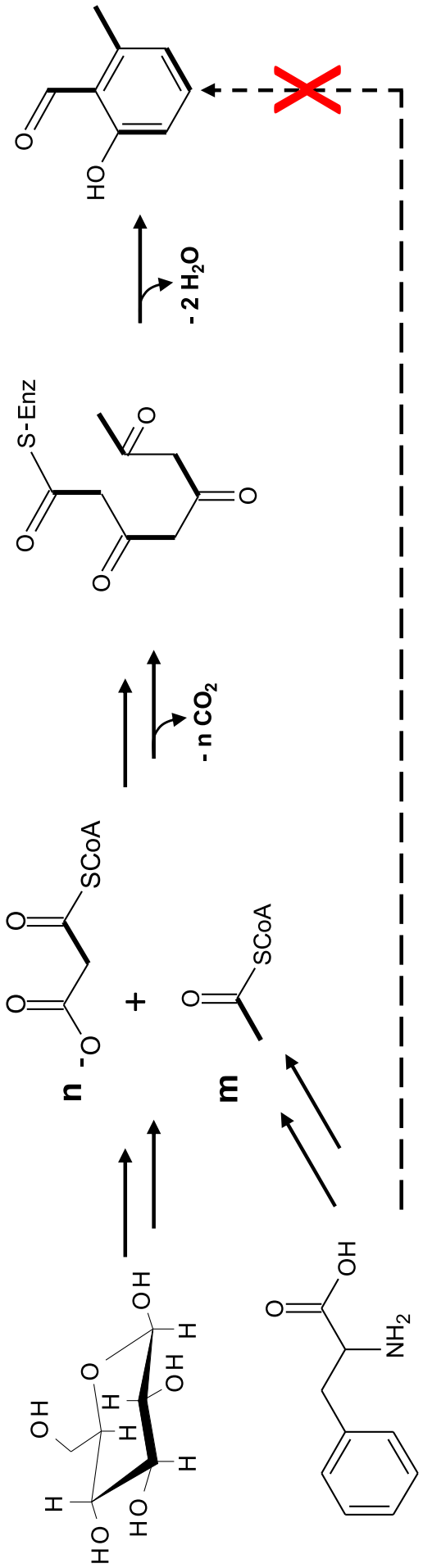
Table heading

Table 1. Enrichment factors of the respective ions in treatment groups compared to the control calculated based on mass spectrometry. The mass/charge ratios (*m/z*) 136 and 150 are the molecular ions of 2-hydroxy-6-methyl-benzaldehyde and 3-hydroxybenzene-1,2-dicarbaldehyde, respectively and thus showed no enrichment.

m/z	[$^{13}C_6$, d_7] D-glucose	[$^{13}C_3$] malonic acid	sodium [$^{13}C_1$] acetate	[$^{13}C_6$] phenylalanine
2-hydroxy-6-methyl-benzaldehyde				
136	0	0	0	0
137	8	5	3	3
138	62	38	21	19
139	180	60	17	23
140	269	72	3	23
141	94	25	13	3
142	87	23	14	2
143	86	18	15	1
144	47	17	8	<1
3-hydroxybenzene-1,2-dicarbaldehyde				
150	0	0	0	0
151	4	1	1	2
152	64	20	2	24
153	423	48	14	66
154	2112	151	143	252
155	1170	23	59	96
156	938	15	42	37
157	668	2	30	21
158	737	1	31	1


A
neryl
formate
neral
C13
HO
O
O
HO
O
12.5
13.5
14.5
15.5
(min)
B
ns
[%] aromatic compounds
20
15
10
5
0
C
*
bacteria/host copy ratio (n*10³)
30
22.5
15
7.5
0
control
antibiotics
D
E
F
G


control (wheat grass)
100
0
136
77
122
93
80
90
100
110
120
130
140
150
m/z
relative abundance (%)
[13C6, d7]-D glucose + antibiotics + wheat grass
100
0
136
77
93
122
144
80
90
100
110
120
130
140
150
m/z


- 2 H2O
- n CO2
S-Enz
SCoA
SCoA
n
m
NH2

10.4 Food selection

10.4.1 Publication 7: **Track the snack – olfactory cues shape foraging behaviour of decomposing soil mites (Oribatida)**

Adrian Brückner, Romina Schuster, Timo Smit, Melanie M. Pollierer, Irmgard Schäffler and Michael Heethoff

Published in: Pedobiologia 66: 74-80

Submitted: 07.10.2017, Accepted: 31.10.2017, Published: 05.02.2018

Authors contributions:

MH and AB designed the research; RS, TS and AB performed bioassays; AB and IS performed chemical analyses; MMP contributed materials; AB analyzed the data; AB and MH wrote the paper. All authors read, discussed and approved the final version.

Reprinted by permission from Elsevier. Pedobiologia – Journal of Soil Ecology. Track the snack – olfactory cues shape foraging behaviour of decomposing soil mites (Oribatida). Brückner Adrian, Schuster Romina, Smit Timo, Pollierer M. Melanie, Schäffler Irmgard, Heethoff Michael. © Elsevier Ltd. 2018

Pedobiologia - Journal of Soil Ecology 66 (2018) 74–80

Contents lists available at ScienceDirect

Pedobiologia - Journal of Soil Ecology

journal homepage: www.elsevier.com/locate/pedobi

Track the snack – olfactory cues shape foraging behaviour of decomposing soil mites (Oribatida)

Adrian Brückner[a,*], Romina Schuster[a], Timo Smit[a], Melanie M. Pollierer[b], Irmgard Schäffler[c], Michael Heethoff[a,*]

[a] Ecological Networks, Technische Univeristät Darmstadt, Schnittspahnstraße 3, 64287 Darmstadt, Germany
[b] J.F Blumenbach Institute of Zoology and Anthropology, University of Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany
[c] Department of Ecology & Evolution, University of Salzburg, Hellbrunnerstraße 34, 5020 Salzburg, Austria

ARTICLE INFO

Keywords:
Chemical ecology
Soil food webs
Olfaction
Gas chromatography/mass spectrometry
Fungal volatiles
Feeding ecology
Choosy generalist

ABSTRACT

Olfactory cues are important for many heterotrophic organisms to find and identify food. In soil ecosystems, however, olfactory food selection has only been fragmentarily explored and it remains to be uncovered whether olfactory signals are involved in finding suitable food sources in microarthropods. We addressed this basic question in laboratory food-choice bioassays with two oribatid mite species [*Archegozetes longisetosus* (opportunistic feeder) and *Scheloribates* sp. (myco-/phytophagous feeder)] and bacteria, fungi, lichen and litter as resources. We found that both oribatid mite species used olfactory cues to find and differentiate among food resources. While *A. longisetosus* preferred bacterial diet, *Scheloribates* sp. mainly fed on fungal-based food sources (fungi and lichen). We analysed volatiles and nutrients of the resources and used modified food, synthetic compounds and amino/fatty acid mixtures in a second food-choice experiment. *A. longisetosus* preferred fatty acids (and their preferred bacterial diet was also rich in fatty acids), while *Scheloribates* was highly attracted by mushroom-alcohol (1-octen-3-ol). Our experiments showed that olfactory signals contribute to the trophic ecology of oribatid mites in soil ecosystems.

1. Introduction

Foraging processes of arthropods in complex habitats like soils rely on different tactile perception mechanisms, but also chemical cues could be involved (Bengtsson et al., 1991; Hedlund et al., 1995; Hall and Hedlund, 1999; Zirbes et al., 2011). Such stimuli enable soil organisms to localize and distinguish food sources in an opaque and a highly heterogeneous environment with patchy resource distribution (Anderson, 1975a; Burnett et al., 1998; Cain et al., 1999; Farley and Fitter, 1999; Hodge, 2006; Nielsen et al., 2010). Due to this patchiness, resource and habitat specialization appear to be rare in detritivorous soil arthropods such as springtails or oribatid mites, and it is assumed that most species predominantly feed on a broad food spectrum (Luxton, 1972; Anderson, 1975b; Behan-Pelletier and Hill, 1983; Walter, 1987; Vegter et al., 1988; Ettema and Wardle, 2002; Wehner et al., 2016). However, in contrast to this generally low degree of resource partitioning, stable isotope analyses indicate a more distinct feeding niche (Schneider et al., 2004a; Chahartaghi et al., 2005; Pollierer et al., 2009). Hence, soil detritivores have been termed "selective generalist feeders" or "choosy generalists" (Klironomos et al., 1992; Schneider and Maraun, 2005). Accordingly, it seems advantageous to have a general, olfactory based foraging strategy to recognize and find food in the first place, while gustatory properties or structure/morphology may facilitate the final choice to consume a resource. For "choosy generalist" feeders, it should be beneficial to use a broad spectrum of volatiles to locate food, instead of solely depending on one specific signal (Hedlund et al., 1995; Ferry et al., 2007; Pfeffer and Filser, 2010).

However, little is known about olfactory mediated foraging and food selection by soil microarthropods and soil-dwelling predatory larvae (Thomas et al., 2008). In pioneer studies Bengtsson et al. (1988, 1991) and Hedlund et al. (1995) have shown that springtails (Collembola) use fungal volatiles to perceive and choose among fungal resources, but are also attracted to odorants of other animals (e.g., Salmon and Ponge, 2001; Nilsson and Bengtsson, 2004). Thus, springtails are also able to sense and respond to fungal species with different levels of toxicity (i.e. secondary metabolites) using olfactory signals (Staaden et al., 2011; Stötefeld et al., 2012). Furthermore, springtails are differently attracted to sex-specific volatiles of mosses, can locate soil patches with high microbial activity and seem to move towards CO_2

* Corresponding authors.
E-mail addresses: adrian.brueckner@gmail.com (A. Brückner), heethoff@bio.tu-darmstadt.de (M. Heethoff).

http://dx.doi.org/10.1016/j.pedobi.2017.10.004
Received 7 October 2017; Received in revised form 31 October 2017; Accepted 31 October 2017
0031-4056/ © 2017 Elsevier GmbH. All rights reserved.

sources (Moursi, 1962; Hassall et al., 1986; Rosenstiel et al., 2012). For (soil) mites, however, the olfactory base of food selection and responses to resource derived volatiles are poorly understood. For example, only few studies showed that predatory mites seem to respond to herbi-/fungivore induced volatiles of their prey (Hall and Hedlund, 1999; Aratchige et al., 2004; Pfeffer and Filser, 2010), and stored-product mites (Actinotrichida, Astigmata) are attracted by olfactory stimuli, mostly mixtures of semi-volatile amino- and fatty acids (Pankiewicz-Nowicka et al., 1986, 1987). Compared to above-ground invertebrates, the movement patterns of soil-dwellers are less directional and olfactory signals are presumably only detected over a few centimetres (Auclerc et al., 2010). Also the rather enigmatic nature of the below-ground-system, i.e. the heterogeneity and patchiness of the habitat, the multi-layered structure of soil, litter and root system (e.g., Anderson 1975a; Hodge, 2006; Nielsen et al., 2010), but also the less developed receptor systems (Wu et al., 2017) may render soil arthropods less directional compared to their above-ground relatives. For example, collembolans exhibit irregular, non-directional walks and also systematic loop-searching behaviour at higher distances, but switch to directional movement if an olfactory source is nearby (Bengtsson et al., 2004; Auclerc et al., 2010).

Here we focus on oribatid mites – highly diverse soil-dwelling mites abundant in various ecosystems all over the world (Schatz, 2004; Schatz et al., 2011). Niche partitioning and especially food preferences have been intensively studied in oribatid mites (Schneider et al., 2004b; Maraun et al., 2011). Based on available data on stable isotope composition ($\delta^{15}N/\delta^{13}C$), oribatid mites can be classified as lichen feeders, as primary as well as secondary decomposers and predators/scavengers (Schneider et al., 2004a; Maraun et al., 2011). Whereas primary decomposers mainly feed on plant material, secondary decomposers are considered as microbivores, i.e. feeding on algae, bacteria and fungi (Schuster, 1956; Luxton, 1972). Also gut boli and fecal pellet analyses (Anderson, 1975b; Labandeira et al., 1997), cheliceral morphology (Kaneko, 1988; Perdomo et al., 2012), enzymology (Siepel and de Ruiter-Dijkman, 1993; Hubert et al., 2001), molecular gut bar-coding (Heidemann et al., 2011; Eitzinger et al., 2013) and especially laboratory preference tests demonstrated a broad resource spectrum with distinct preferences for certain leaf-litter material, algae or fungi (Pande and Berthet, 1973; Hubert and Lukesova, 2001; Schneider et al., 2005; Koukol et al., 2009). However, the actual mechanism enabling food selection is unknown. On numerous occasions we observed that mites in our laboratory cultures, although being blind, often quickly and directionally head towards their food. Thus, we asked ourselves how olfactory senses could be involved in this behaviour.

Generally, responses to volatile cues in oribatid mites are known in the context of chemical communication (Shimano et al., 2002; Raspotnig, 2006; Heethoff and Raspotnig, 2012). For example the giant moss mite (*Collohmannia gigantea*) uses a multicomponent gland secretion for chemical defense, which has evolved a secondary function as alarm pheromone (Raspotnig, 2006). Olfactory associated sensory organs, the solenidia, i.e. setea with wall-porous structures and branched dendrites, are found on the tarsi of oribatid mites (Baker, 1985; Alberti, 1998; De Lillo et al., 2004). Furthermore, most soil-dwelling oribatid mites do not possess structures to perceive and process visual information. Some kind of light-sensitive organs (i.e., lenticuli) are, however, present in some Enarthronota and Brachypylina, but absent from *Archegozetes* and *Scheloribates* (e.g. Alberti and Fernandez, 1988, 1990). Hence, it seems most likely that soil dwelling oribatid mites also use their olfactory sense to localize chemicals associated with food.

We used two oribatid mite species (the desmonomatan *Archegozetes longisetosus* and the brachypyline *Scheloribates* sp.) to investigate whether oribatid mites can use olfactory cues for food localization and discrimination, and if so, which chemical substances may serve as candidate olfactory signals responsible for food choice.

2. Materials and methods

2.1. Mites

Archegozetes longisetosus Aoki (a middle-derivative desmonomatan species) is a common parthenogenetic (=all-female), widely distributed pantropical mite and an opportunistic feeder (Heethoff and Scheu, 2016). The laboratory strain *A. longisetosus* ran (Heethoff et al., 2007) has been used as a model organism for more than 20 years (Heethoff et al., 2013). *Scheloribates* Berlese (a highly derived brachypyline genus) is a sexually reproducing myco- and phytophagous feeder (Siepel, 1996; Hubert and Lukesova, 2001). The unidentified species used in this study is probably tropical, since we obtained it as a contamination of a tropical springtail culture send in coconut debris from Zoo Zajac (Duisburg, Germany). Both oribatid mite species were raised in polypropylene boxes, where the bottom was covered with a mixture of plaster of Paris: activated charcoal (9:1). The stock cultures were kept humid at 28 °C in darkness. *A. longisetosus* cultures were fed with lupine flour (Govinda Natur GmbH, Neuhofen, Germany), while *Scheloribates* sp. was fed with yeast (Rapunzel Naturkost GmbH, Legau, Germany).

2.2. Food and nutrient mixtures

To test olfactory food choice of the two oribatid mite species we prepared different semi-natural and artificial foods/mixtures. As semi-natural resources we chose the gram-negative soil bacterium *Pseudomonas fluorescens* CHA19, the mesophilic soil fungus *Chaetomium globosum*, the fruticose lichen *Cladonia rangiformis* and fallen leaf litter of *Tilia cordata*. *Pseudomonas fluorescens* CHA19 (hereafter: bacteria) was cultivated in liquid modified arabinose-gluconate medium (Cole and Elkan, 1973; van Berkum, 1990) without amino acids. *Chaetomium globosum* (hereafter: fungi) was grown on cellophane over Czapek- Dox agar (Warcup, 1950) plates. *Cladonia rangiformis* (hereafter: lichen) was collected in November 2016 on a dry, sandy meadow in the botanical garden in Darmstadt (49°52′10.4″N 8°40′48.1″E). *Tilia cordata* (hereafter: litter) leaf litter was collected in autumn 2013 in an old lime forest with mainly *Tilia cordata*, interspersed with some common oak (*Quercus robur*) and bordered by sycamore (*Acer pseudoplatanus*) and ash (*Fraxinus excelsior*) near Duderstadt (51°30′21.1″N 10°12′27.5″E). Bacteria, fungi and lichen material was freeze-dried over a period of 72 h, whereas litter was dried at 50 °C for two days. Subsequently, all four food sources were ground and stored dry until the experiment started. We also prepared five artificial nutrient mixtures consisting of only essential amino acids (mixture of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine in equal proportions), non-essential amino acids (mixture of alanine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, proline, serine and tyrosine in equal proportions), fatty acids (C16:0, C18:0, C18:1Δ9, C18:2Δ6,9; mixing ratio (mass based) 1:1:0.5:0.1), pure D-glucose (99.9%) and D-glucose mixed with mushroom alcohol (1-octen-3-ol; 1 µl/1 g glucose). We used these mixtures because other sarcoptiform mites (Astigmata) appear to react and discriminate among these compounds (Pankiewicz-Nowicka et al., 1986, 1987). All nutrients/chemicals were analytical grade and purchased from Sigma-Aldrich, Darmstadt, Germany.

2.3. Experimental setup

Experiments were performed in plastic Petri dishes (4.5 × 1.5 cm or 9 × 1.5 cm, for the olfactory sensing test or choice experiment I–III, respectively) grounded with moist analytical filter paper (both, Hartenstein GmbH, Würzburg, Germany). Four different powders (either semi-natural food or nutrient mixtures, except for the olfactory

sensing test) were placed marginally in a circle to maximize the distances to each other. We used recently eclosed adult mites (< 1 week) that had never experienced the resources powders before and were only used for one experiment to ensure naïve animals. Ten specimens were placed in the middle of the Petri dish for every replicate, and after one hour (for the choice experiments) or 10 min (for the olfactory sensing test) mites within < 2 mm distance to the powder patches were counted. Individuals which were not found near a resource (> 2 mm or no movement) were counted as "wanderers" and excluded from statistical analyses. All experiments were conducted at 25 ± 2 °C in darkness. The behavioural tests were statistically analysed (details see below) with R 3.3.1 "Bug in Your Hair" (R Core Team, 2016).

2.3.1. Olfactory sensing test

To test if mites could generally sense food using olfactory cues, ten specimens of *A. longisetosus* (240 specimens; n = 24 assays) were placed in the middle of small plastic Petri dishes. On one site we offered a portion of dry yeast, on the other side we provided a small ball of moist filter paper. To ensure that food was found based on olfactory cues rather than on random exploration of the arenas, we visually inspected that mites moved directly towards the resources and counted the specimens sitting on either yeast or filter-paper balls after 10 min. These counts were statistically analysed using a Wilcoxon signed-rank test with experimental ID as blocking factor.

2.3.2. Choice test I: resource assay

We offered bacteria, fungi, lichen and litter to *A. longisetosus* (1680 specimens; n = 168 assays) and *Scheloribates* sp. (960 specimens; n = 96 assays). Overall olfactory preferences were analysed as oribatid mite counts for each powder using Friedman test with experimental ID as blocking factor, while Wilcoxon signed-rank tests with affiliated false-discovery rate correction (Benjamini and Hochberg, 1995) were used for pairwise comparisons of differences among the four resource powders. When all resources are equally attractive, we expect an equal distribution on all kinds of food (25% on each). Significant deviations were interpreted as the resource being less attractive (< 25%) or preferred (> 25%).

2.3.3. Choice test II: nutrient assay

We offered glucose, essential amino acids, non-essential amino acid and fatty acid mixtures to *A. longisetosus* (960 specimens; n = 96 assays) and *Scheloribates* sp. (960 specimens; n = 96 assays). Overall olfactory preferences were analysed as described in choice test I.

2.3.4. Choice test III: mushroom alcohol assay

We offered glucose infused with mushroom alcohol, the essential amino acid, the non-essential amino acid and the fatty acid mixtures to *A. longisetosus* (960 specimens; n = 96 assays) and *Scheloribates* sp. (960 specimens; n = 96 assays). Overall olfactory preferences were analysed as described in choice test I.

2.4. Volatile and nutrient analyses

2.4.1. Volatile collection and analyses

Thermal desorption (TD) samples of the four resource powders were collected using dynamic headspace methods as described by Dötterl et al. (2005); for more details see Supplementary information. TD-samples (n = 2) were analysed using a Shimadzu GC/MS-QP2010 Ultra (GC equipped with a ZB-5MS fused silica capillary column; length: 60 m × 0.25 mm, df = 0.25 µm, from Phenomenex, Aschaffenburg, Germany) equipped with an automatic TD system (TD-20, Shimadzu, Japan); for details see Braunschmid et al. (2017). Identification of scent components was carried out by checking and interpreting the mass spectra accompanied by using the mass spectral data bases NIST 11, Wiley 9, MassFinder 3, FFNSC 2, and Adams (2007). Whenever possible, components were verified using retention indices and mass spectra of authentic standards or by comparison with published retention indices (Van den Dool and Kratz, 1963). Contaminates were excluded (based on the negative control) and the mean compositional data (in%) was subjected to cluster analysis using UPGMA on Bray-Curtis similarities [= BCS (Bray and Curtis, 1957)] in PAST 3.13 (Hammer et al., 2001).

2.4.2. Fatty acids

Lipids were extracted from the bacteria/fungi/lichen/litter powders (25 ± 5 mg dry weight; four replicates each) using 1 ml of a chloroform:methanol-mixture, 2:1 (V/V) according to Folch et al. (1957) over a period of 24 h. Afterwards two replicate extracts were purified and separated using column chromatography according to the methods described by Frostegård et al. (1991) and Tserng and Griffin (2003) to fractionate neutral lipid fatty acids (NLFAs) and free fatty acids (FFAs), respectively. Afterwards, the NLFA or FFA containing fractions were derivatized to fatty acid methyl esters (FAMEs) and finally measured with a QP2010 Ultra GC/MS (Shimadzu, Duisburg, Germany) equipped with a ZB-5MS fused silica capillary column (30 m × 0.25 mm ID, df = 0.25 µm) from Phenomenex; for details see Brückner et al. (2017). FAMEs were identified by comparison with analytical standards (37 FAME® and Bacterial Acid Methyl-mixture; Sigma-Aldrich, St. Lois, USA). The fatty acid (i.e. NLFAs and FFAs combined) amounts were quantified with the internal standard (C19; 220 ng/µl) and standardized using the dry weight [mg] of the initial sample.

2.4.3. Amino acids

For analyses of the amino acids, 5 mg (± 0.1 mg) of dry resource powder were diluted in 200 µl of hydrochloric acid (6 mol/l) and boiled for four hours at 100 °C, processed and finally measured as described in Brückner et al. (2017) with an ion exchange chromatograph with ninhydrin post-column derivatization (Biochrom 20+, Amino Acid Analyzer, Cambridge, UK). A standard amino acid mixture (Laborservice Onken GmbH, Gründau, Germany) was used as external standard for quantification. The amount of total amino acids [µg] was standardized using the dry weight [mg] of the initial sample.

2.4.4. C/N ratios

Dried resource powders were weighed into tin capsules (5 ± 1 mg). Total organic carbon and nitrogen contents were measured by an elemental analyser (EA 1108 Elemental Analyser, Carlo Erba, Milan, Italy). Acetanilide (Merck, Darmstadt, Germany) was used as standard. Carbon and nitrogen amounts were calculated based on the standard and the initial dry weight and expressed as C/N ratios.

3. Results

3.1. Olfactory sensing test

Overall, 60% of all individuals made a choice and were included in the analyses – 82 ± 23% (mean ± SD) of *A. longisetosus* chose the dry yeast powder within 10 min, while 18 ± 23% were counted on the moist filter-paper ball (Wilcoxon signed-rank test: $z = 4.01$; $P < 0.0001$; Fig. 1).

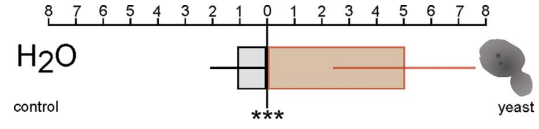


Fig. 1. Number of *Archegozetes longisetosus* counted on either a filter-paper ball (left) or dry yeast powder (right). Bar charts and error bars correspond to mean ± SD. Wilcoxon signed-rank test P < 0.0001 = ***.

Table 1
Mean proportion (± SD) of mites (*Archegozetes longisetosus* and *Scheloribates* sp.) counted on the resources (A), nutrient (B) and mushroom alcohol (C) assays. Different letters within a row indicate significant differences ($P < 0.05$) in pairwise Wilcoxon test comparisons after false discovery rate correction.

A – resource assay	bacteria	fungi	lichen	litter
A. longisetosus	39 ± 23a	24 ± 19b	18 ± 15c	19 ± 18c
Scheloribates sp.	22 ± 21a	38 ± 26b	25 ± 24a	15 ± 17c
B – nutrient assay	**EAS**	**NAS**	**FS**	**Glc**
A. longisetosus	18 ± 18a	15 ± 19a	51 ± 28b	16 ± 18a
Scheloribates sp.	27 ± 26a	25 ± 22a	24 ± 22a	24 ± 23a
C – mushroom alcohol assay	**EAS**	**NAS**	**FS**	**infused Glc**
A. longisetosus	19 ± 16a	14 ± 15ac	52 ± 22b	14 ± 14c
Scheloribates sp.	20 ± 19a	20 ± 17a	21 ± 17a	39 ± 18b

Abbreviations: EAS = essential amino acids; NAS = non-essential amino acids; FS = fatty acids; Glc = D-glucose; modified Glc = D-glucose mixed with 1-octen-3-ol.

3.2. Choice test I: resource assay

A. longisetosus (70% of all individuals included; Friedman-test: $\chi^2 = 58.49$; $P < 0.0001$) and *Scheloribates* sp. (64% of all individuals included; Friedman-test: $\chi^2 = 32.06$; $P < 0.0001$) chose all resources, but showed particular preferences (Table 1). While *A. longisetosus* was most attracted by bacteria-powder and to a lesser extent by fungi-powder, *Scheloribates* sp. preferred fungi-powder, but was also attracted by lichen- and bacteria-powder (Table 1A). Litter-powder was least attractive for both species and *A. longisetosus* also was not attracted by lichen-powder (Table 1A).

3.3. Choice test II: nutrient assay

While *A. longisetosus* showed strong preferences for nutrient mixtures (68% of all individuals included; Friedman-test: $\chi^2 = 49.71$; $P < 0.0001$), *Scheloribates* sp. did not choose any specific mixture (60% of all individuals included; Friedman-test: $\chi^2 = 0.04$; $P = 0.99$; Table 1). The result found for *A. longisetosus* was mostly driven by a strong preference for the fatty acid mixture (Table 1B).

3.4. Choice test III: mushroom alcohol assay

Scheloribates sp. favoured mushroom alcohol (1-octen-3-ol) infused glucose (59% of all individuals included; Friedman-test: $\chi^2 = 54.83$; $P < 0.0001$; Table 1), which did not influence food choice of *A. longisetosus*. Correspondingly, *Scheloribates* sp. preferred 1-octen-3-ol infused glucose over all other nutrient mixtures (Table 1C), while *A. longisetosus* retained favouring the fatty acid mixture (69% of all individuals included; Friedman-test: $\chi^2 = 103.66$; $P < 0.0001$).

3.5. Volatile and nutrient analyses

Overall, we detected 66 different volatile organic compounds (VOCs) of which we could identify 47 (see Supplementary material). Generally, litter-powder had the highest number of different VOCs (50 compounds), whereas bacteria-, lichen- and fungi-powder had 29, 22 and 19, respectively (Fig. 2C). While in total only seven VOCs were shared by all four volatile sources (Fig. 2C), especially litter-powder shared about two-thirds if its compounds with bacteria-, fungi- and lichen-powder (Fig. 2C). Yet, multivariate cluster analysis on BCS (Fig. 2A) revealed that VOC composition of fungi-powder (*Chaetomium globosum*) and lichen-powder (*Cladonia rangiformis*) are most similar followed by litter-powder (*Tilia cordata*) and bacteria-powder (*Pseudomonas flourescens*) as the most distant "chemical group". Each food powder was more specifically characterized by distinct main volatile compounds, namely: 6-methyl-3,5-heptadien-2-one and *cis*-linalool furan oxide for bacteria-powder; 1-octen-3-ol (mushroom alcohol) for fungi-powder; hexan-1-al for lichen-powder and 6-methyl-5-hepten-2-one (sulcatone) for litter-powder (Fig. 2B; see also Supplementary material). Nutrient levels (Figs. 2D–F) overall followed a general pattern; bacteria-powder (C/N = 3.7) and fungi-powder (C/N = 11.8) had low carbon-nitrogen ratios and correspondingly higher amounts of amino acids (bacteria = 129 µg/mg; fungi = 76 µg/mg), whereas especially lichen-powder (C/N = 94.3), but also litter-powder (C/N = 35.4) showed higher C/N ratios and lower amounts of amino acids (lichen = 7.5 µg/mg; litter = 18.5 µg/mg). Additionally, also fatty acid amounts were higher in fungi-powder (20.6 µg/mg) and bacteria-powder (12.2 µg/mg), compared to lower amounts in lichen-powder (7.8 µg/mg) and litter-powder (6.4 µg/mg). The lichen-powder also contained some specific lichen acids, which we did not further identify.

4. Discussion

Overall, our results demonstrated that oribatid mites use chemical cues to find and discriminate food. As expected, *Archegozetes longisetosus* preferred yeast over a negative control (Fig. 1) and only needed a few minutes to localize the food. As observed in other soil microarthropods (i.e. Collembola; Bengtsson et al., 2004; Auclerc et al., 2010) oribatid mites appeared to move straight (see Supplementary material) towards the offered powders [note: the exploratory speed of detritivores is generally slower compared to other arthropods of the same size; Hirt et al. (2017)] supporting a non-random decision. Overall, these observations, and taking into account that most oribatid mites are blind soil-dwellers (Alberti and Fernandez, 1988, 1990), demonstrate that oribatid mites use olfactory senses to recognize and find food.

To further confirm this observational evidence, we firstly conducted bioassays using a set of semi-natural resources present in forest soil systems. Again, we found olfactory guided food preferences in both oribatid mite species. While, *A. longisetosus* preferred bacteria, *Scheloribates* favoured fungal associated food (fungi/lichen); these choices were also underpinned by the chemical analysis of VOCs. Bacterial derived VOC composition was most distant from all the other resources, whereas fungal/lichen VOC compositions were most related with each other. The relatively lower preference of *A. longisetosus* towards lichens (compared to *Scheloribates*) may be related to lichen specific semi-volatile phytochemicals ("lichen acids" and derivates) which were either repellent or attractive for different oribatid mite species in feeding experiments (Reutimann and Scheidegger, 1987). While these experiments tested a gustatory response, our experiments indicate that these chemical cues also mediate/alter olfactory based food choice, especially since lichen acids and their combustion products have been characterized as repellent or even toxic for insects (Lange, 1957; Emmerich et al., 1993; Cocchietto et al., 2002; Nimis and Skert, 2006). Yet all resources shared a certain amount of VOCs (Fig. 2C), which may explain the relatively generalistic resource choice of both mite species and confirm the "choosy generalists" concept (Schneider and Maraun, 2005). Our results demonstrate that this conceptual idea may be explained by olfactory based/guided food selection and discrimination. For example, the genus *Scheloribates* is known to be myco-/phytophagous (Siepel, 1996; Hubert and Lukesova, 2001) and interestingly the olfactory food choice in our experiment matched this.

Secondly, we tested the olfactory response of oribatid mites towards artificial mixtures of the three most important macronutrients (amino acids, fatty acids and sugar), because also these substances seem to be attractive for soil animals and insects, even if the volatility is comparatively low (e.g., Morton and Bateman, 1981; Pankiewicz-Nowicka

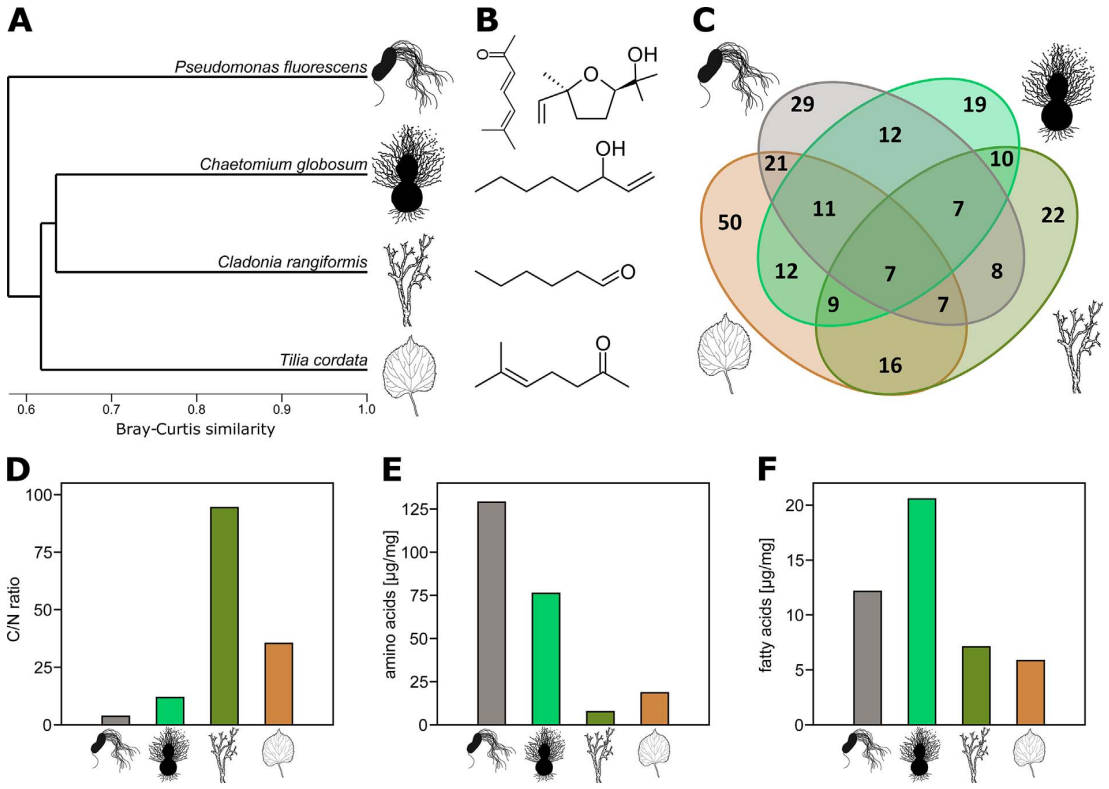


Fig. 2. Similarity (A) of volatile organic compound (VOC) profiles of *Pseudomonas fluorescens, Chaetomium globosum, Cladonia rangiformis* and *Tilia cordata* displayed in a cluster dendrogram (using UPGMA on Bray-Curtis similarities). Chemical structures (B) of the most abundant VOCs and number of shared VOCs displayed in a Venn diagram (C) of the resources. Summarized nutrient data of the four offered semi-natural resources powders; the carbon/nitrogen-ratio (C/N ratio; D), the amounts of amino (E) and fatty acids (F). Resources pictograms correspond to the names in (A).

et al., 1986, 1987; Salmon and Ponge, 2001). Furthermore, the mites' response towards macronutrient mixtures may serve as a proxy to understand their different preferences for the food powders in our experiment. We found that *A. longesitosus* preferred fatty acids, while *Scheloribates* showed no preferences for any mixture. Interestingly, bacteria contained the second highest amount of NLFAs and the highest amount of FFAs (see Fig. 2) of all tested resources and therefore fatty acids (and fatty acids derived VOCs) are potentially important olfactory cues for *A. longisetosus*. Also generalistic pest mites of the cohort Astigmata (more specifically: dried-fruit mites, grain mites and mold mites) showed preferences towards longer-chained fatty acids in choice-bioassays (Pankiewicz-Nowicka et al., 1986, 1987). Since *Scheloribates* is myco-/phytophagous and since most fungi emit specific fungal VOCs (Hung et al., 2015) we thirdly infused D-glucose with mushroom alcohol (1-octen-3-ol). As expected, *Scheloribates* sp. subsequently started to favor the infused glucose, whereas *A. longisetosus* did not change its behaviour. Mushroom alcohol was also the most abundant VOC in fungi and the second most abundant in lichen (Fig. 2B, Supplementary data), and thus mushroom alcohol seem to modify *Scheloribates* sp. response to food resources. In this study we only focused on the most abundant VOC (mushroom alcohol) and the macronutrients to study basic mechanisms of olfactory resources choice in detritivores mites, yet comparative studies on VOCs from different sources and their combinations promise to reveal causes and consequences of foraging pattern found in soil dwelling mites.

Some general hypotheses about olfaction and food choice in oribatid mites arise from our experiments: the generalist detritivore reacted to olfactory signals of distinct food sources and appeared to use unspecific signals (e.g., fatty acids), whereas more specialized feeders (e.g., fungivores) preferred more specific VOCs (e.g., mushroom alcohol) to localize food. Furthermore, olfactory based preferences raise the question if food odor bouquets also include intrinsic information on macronutrients or biosynthetically derived compounds ("nutritional cues"). This may or may not be the case for oribatid mites, since the preferred resources (bacteria and fungi) also contained most fatty- and amino acids (Fig. 2E–F). However, *Scheloribates* sp. showed a certain preference for lichen which was the nutritionally poorest food (see C/N ratios, Fig. 2D). Thus, there seems to be no obvious evidence for "nutritional cues" in olfactory signaling in oribatid mites so far, but our experiments gave first hints on different signal types for generalist/specialist oribatid mites.

Ethical statement

There are no legal restrictions on working with mites.

Authors' contributions

MH and AB designed the research; RS, TS and AB performed bioassays; AB and IS performed chemical analyses; MMP contributed materials; AB analysed the data; AB and MH wrote the paper. All authors read, discussed and approved the final version.

Competing financial interests

The authors declare no competing financial interests.

Acknowledgments

We acknowledge the support of Markus Geißler, Andrea Hilpert, Ursula Lebong, Stefan Schneckenburger, Christian Storm and Jascha Volk. We are grateful to Valerie Behan-Pelletier and Roy A. Norton for their help with obtaining literature. AB was supported by the German Nation Academic Foundation (Studienstiftung des deutschen Volkes). This study was supported by the German Science Foundation (DFG; HE 4593/5-1).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.pedobi.2017.10.004.

References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/ mass Spectrometry. Allured Publishing Corporation, Carol Stream.
Alberti, G., Fernandez, N.A., 1988. Fine structure of a secondarily developed eye in the freshwater moss-mite, *Hydrozetes lemnae* (Coggi, 1899)(Acari: Oribatida). Protoplasma 146, 106–117.
Alberti, G., Fernandez, N.A., 1990. Aspects concerning the structure and function of the lenticulus and clear spot of certain oribatids (Acari, Oribatida). Acarologia 31, 65–72.
Alberti, G., 1998. Fine structure of receptor organs in oribatid mites (Acari). In: Ebermann, E. (Ed.), Arthropod Biology: Contributions to Morphology, Ecology and Systematics. Austrian Academy of Sciences Press, Vienna, pp. 27–77.
Anderson, J.M., 1975a. The enigma of soil animal diversity. In: Vanek, J. (Ed.), Proceedings of the 5th International Colloquium on Soil Zoology. Prague September 17–22, 1973. Academia Prague. pp. 51–58.
Anderson, J.M., 1975b. Succession, diversity and trophic relationships of some soil animals in decomposing leaf litter. J. Anim. Ecol. 44, 475–495.
Aratchige, N.S., Lesna, I., Sabelis, M.W., 2004. Below-ground plant parts emit herbivore-induced volatiles: olfactory responses of a predatory mite to tulip bulbs infested by rust mites. Exp. Appl. Acarol. 33, 21–30.
Auclerc, A., Libourel, P.A., Salmon, S., Bels, V., Ponge, J.F., 2010. Assesment of movement patterns in *Folsomia candida* (Hexapoda: Collembola) in the presence of food. Soil Biol. Biochem. 42, 657–659.
Baker, G.T., 1985. Morphology of the solenidia and famulus on tarsi I and II of *Rhizoglyphus robini* Calaparède (Acaridae). Zoologische Jahrbücher. Abteilung für Anatomie und Ontogenie der Tiere 113. pp. 85–89.
Behan-Pelletier, V.M., Hill, S.B., 1983. Feeding-habits of sixteen species of oribatei (Acari) from an acid peat bog glenamoy, Ireland. Revue D Ecologie Et De Biologie Du Sol 20, 221–267.
Bengtsson, G., Erlandsson, A., Rundgren, S., 1988. Fungal odor attracts soil Collembola. Soil Biol. Biochem. 20, 25–30.
Bengtsson, G., Hedlund, K., Rundgren, S., 1991. Selective odor perception in the soil Collembola *Onychiurus armatus*. J. Chem. Ecol. 17, 2113–2125.
Bengtsson, G., Nilsson, E., Ryden, T., Wiktorsson, M., 2004. Irregular walks and loops combines in small-scale movement of a soil insect: implications for dispersal biology. J. Theor. Biol. 231, 299–306.
Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate – a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B-Methodol. 57, 289–300.
Brückner, A., Hilpert, A., Heethoff, M., 2017. Biomarker function and nutritional stoichiometry of neutral lipid fatty acids and amino acids in oribatid mites. Soil Biol. Biochem. 115, 35–43.
Braunschmid, H., Mükisch, B., Rupp, T., Schäffler, I., Zito, P., Birtele, D., Dötterl, S., 2017. Interpopulation variation in pollinators and floral scent of the lady's-slipper orchid *Cypripedium calceolus* L. Arthropod-Plant Interact. 1–17.
Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27, 326–349.
Burnett, M.R., August, P.V., Brown, J.H., Killingbeck, K.T., 1998. The influence of geomorphological heterogeneity on biodiversity I. A patch-scale perspective. Conserv. Biol. 12, 363–370.
Cain, M.L., Subler, S., Evans, J.P., Fortin, M.J., 1999. Sampling spatial and temporal variation in soil nitrogen availability. Oecologia 118, 397–404.
Chahartaghi, M., Langel, R., Scheu, S., Ruess, L., 2005. Feeding guilds in Collembola based on nitrogen stable isotope ratios. Soil Biol. Biochem. 37, 1718–1725.
Cocchietto, M., Skert, N., Nimis, P.L., Sava, G., 2002. A review on usnic acid, an interesting natural compound. Naturwissenschaften 89, 137–146.
Cole, M.A., Elkan, G.H., 1973. Transmissible resistance to penicillin G, neomycin, and chloramphenicol in *Rhizobium japonicum*. Antimicrob. Agents Chemother. 4, 248–253.
De Lillo, E., Nuzzaci, G., Di Palma, A., 2004. Sensorial structures in mites and perspectives of research. Phytophaga 14, 59–81.
Dötterl, S., Wolfe, L.M., Jürgens, A., 2005. Qualitative and quantitative analyses of flower scent in *Silene latifolia*. Phytochemistry 66, 203–213.
Eitzinger, B., Micic, A., Körner, M., Traugott, M., Scheu, S., 2013. Unveiling soil food web links: new PCR assays for detection of prey DNA in the gut of soil arthropod predators. Soil Biol. Biochem. 57, 943–945.
Emmerich, R., Giez, I., Lange, O.L., Proksch, P., 1993. Toxicity and antifeedant activity of lichen compounds against the polyphagous herbivorous insect *Spodoptera littoralis*. Phytochemistry 33, 1389–1394.
Ettema, C.H., Wardle, D.A., 2002. Spatial soil ecology. Trends Ecol. Evol. 17, 177–183.
Farley, R.A., Fitter, A.H., 1999. The responses of seven co-occurring woodland herbaceous perennials to localized nutrient-rich patches. J. Ecol. 87, 849–859.
Ferry, A., Dugravot, S., Delattre, T., Christides, J.P., Auger, J., Bagneres, A.G., Poinsot, D., Cortesero, A.M., 2007. Identification of a widespread monomolecular odor differentially attractive to several *Delia radicum* ground-dwelling predators in the field. J. Chem. Ecol. 33, 2064–2077.
Folch, J., Lees, M., Sloane-Stanley, G., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.
Frostegård, A., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. J. Microbiol. Methods 14, 151–163.
Hall, M., Hedlund, K., 1999. The predatory mite *Hypoaspis aculeifer* is attracted to food of its fungivorous prey. Pedobiologia 43, 11–17.
Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontol. Electronica 4, 9.
Hassall, M., Visser, S., Parkinson, D., 1986. Vertical migration of *Onychiurus subtenuis* (Collembola) in relation to rainfall and microbial activity. Pedobiologia 29, 175–182.
Hedlund, K., Bengtsson, G., Rundgren, S., 1995. Fungal odour discrimination in two sympatric species of fungivorous collembolans. Funct. Ecol. 9, 869–875.
Heethoff, M., Raspotnig, G., 2012. Investigating chemical communication in oribatid and astigmatid mites in bioassays – pitfalls and suggestions. Soil Org. 84, 409–421.
Heethoff, M., Scheu, S., 2016. Reliability of isotopic fractionation (△15N, △13C) for the delimitation of trophic levels of oribatid mites: diet strongly affects △13C but not (15N. Soil Biol. Biochem. 101, 124–129.
Heethoff, M., Laumann, M., Bergmann, P., 2007. Adding to the reproductive biology of the parthenogenetic oribatid mite, *Archegozetes longisetosus* (Acari, Oribatida, Trhypochthoniidae). Turk. J. Zool. 31, 151–159.
Heethoff, M., Bergmann, P., Laumann, M., Norton, R.A., 2013. The 20th anniversary of a model mite: a review of current knowledge about *Archegozetes longisetosus* (Acari, Oribatida). Acarologia 53, 353–368.
Heidemann, K., Scheu, S., Ruess, L., Maraun, M., 2011. Molecular detection of nematode predation and scavenging in oribatid mites: laboratory and field experiments. Soil Biol. Biochem. 43, 229–236.
Hirt, M.R., Lauermann, T., Brose, U., Noldus, L.P.J.J., Dell, A.I., 2017. The little things that run: a general scaling of invertebrate exploratory speed with body mass. Ecology. http://dx.doi.org/10.1002/ecy.2006.
Hodge, A., 2006. Plastic plants and patchy soils. J. Exp. Bot. 57, 401–411.
Hubert, J., Lukesova, A., 2001. Feeding of the panphytophagous oribatid mite *Scheloribates laevigatus* (Acari: Oribatida) on cyanobacterial and algal diets in laboratory experiments. Appl. Soil Ecol. 16, 77–83.
Hubert, J., Zilova, M., Pekar, S., 2001. Feeding preferences and gut contents of three panphytophagous oribatid mites (Acari: Oribatida). Eur. J. Soil Biol. 37, 197–208.
Hung, R., Lee, S., Bennett, J.W., 2015. Fungal volatile organic compounds and their role in ecosystems. Appl. Microbiol. Biotechnol. 99, 3395–3405.
Kaneko, N., 1988. Feeding habits and cheliceral size of oribatid mites in cool temperate forest soils in Japan. Revue d'Ecologie et de Biologie du Sol 25, 353–363.
Klironomos, J.N., Widden, P., Deslandes, I., 1992. Feeding preferences of the Collembolan *Folsomia candida* in relation to microfungal succession on decaying litter. Soil Biol. Biochem. 24, 685–692.
Koukol, O., Mourek, J., Janovsky, Z., Cerna, K., 2009. Do oribatid mites (Acari: Oribatida) show a higher preference for ubiquitous vs. specialized saprotrophic fungi from pine litter? Soil Biol. Biochem. 41, 1124–1131.
Labandeira, C.C., Phillips, T.L., Norton, R.A., 1997. Oribatid mites and the decomposition of plant tissues in Paleozoic coal-swamp forests. Palaios 12, 319–353.
Lange, O.L., 1957. Die flechte *Parmelia paraguariensis* als handelsware in Südlichen Sahara. Natur und Volk 87, 266–273.
Luxton, M., 1972. Studies on oribatid mites of a Danish beech wood soil.1. Nutritional biology. Pedobiologia 12, 434–463.
Maraun, M., Erdmann, G., Fischer, B.M., Pollierer, M.M., Norton, R.A., Schneider, K., Scheu, S., 2011. Stable isotopes revisited: their use and limits for oribatid mite trophic ecology. Soil Biol. Biochem. 43, 877–882.
Morton, T.C., Bateman, M.A., 1981. Chemical studies on proteinaceous attractants for fruit flies, including the identification of volatile constituents. Aust. J. Agric. Res. 32, 905–916.
Moursi, A.A., 1962. The attractiveness of CO_2 and N_2 to soil arthropoda. Pedobiologia 1, 299–302.
Nielsen, U.N., Osler, G.H.R., Campbell, C.D., Neilson, R., Burslem, D.F.R.P., van der Wal, R., 2010. The enigma of soil animal species diversity revisited: the role of small-scale heterogeneity. PLoS One 5, e11567.
Nilsson, E., Bengtsson, G., 2004. Death odour changes movement pattern of a Collembola. Oikos 104, 509–517.
Nimis, P.L., Skert, N., 2006. Lichen chemistry and selective grazing by the coleopteran *Lasioderma serricorne*. Environ. Exp. Bot. 55, 175–182.
Pande, Y.D., Berthet, P., 1973. Studies on the food and feeding habits of soil Oribatei in a black pine plantation. Oecologia 12, 413–426.
Pankiewicz-Nowicka, D., Boczek, J., Davis, R., 1986. Attraction by selected organic compounds to *Tyrophagus putrescentiae* (Acari: Acaridae). Ann. Entomol. Soc. Am. 79, 293–299.
Pankiewicz-Nowicka, D., Boczek, J., Davis, R., 1987. Attraction of *Caropoglyphus lactis* (Acarina: Acaridae) to selected organic compounds. Exp. Appl. Acarol. 3, 307–315.
Perdomo, G., Evans, A., Maraun, M., Sunnucks, P., Thompson, R., 2012. Mouthpart morphology and trophic position of microarthropods from soils and mosses are strongly correlated. Soil Biol. Biochem. 53, 56–63.

Pfeffer, S.P., Filser, J., 2010. Attraction to prey and prey-associated odours by the predatory mite *Hypoaspis aculeifer* in a soil experimental system. Soil Biol. Biochem. 42, 1355–1357.
Pollierer, M.M., Langel, R., Scheu, S., Maraun, M., 2009. Compartmentalization of the soil animal food web as indicated by dual analysis of stable isotope ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$). Soil Biol. Biochem. 41, 1221–1226.
R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria ISBN 3-900051-07-0. htttp://www.R-project.org.
Raspotnig, G., 2006. Chemical alarm and defence in the oribatid mite *Collohmannia gigantea* (Acari: Oribatida). Exp. Appl. Acarol. 39, 177–194.
Reutimann, P., Scheidegger, C., 1987. Importance of lichen secondary products in food choice of two oribatid mites (Acari) in an alpine meadow ecosystem. J. Chem. Ecol. 13, 363–369.
Rosenstiel, T.N., Shortlidge, E.E., Melnychenko, A.N., Pankow, J.F., Eppley, S.M., 2012. Sex-specific volatile compounds influence microarthropod-mediated fertilization of moss. Nature 489 431-U118.
Salmon, S., Ponge, J.F., 2001. Earthworm excreta attract soil springtails: laboratory experiments on *Heteromurus nitidus* (Collembola: Entomobryidae). Soil Biol. Biochem. 33, 1959–1969.
Schatz, H., Behan-Pelletier, V.M., O'Connor, B.M., Norton, R.A., 2011. Suborder Oribatida van der Hammen, 1968. In: Zhang, Z.-Q. (Ed.), Animal Biodiversity: An Outline of Higher-level Classification and Survey of Taxonomic Richness. Zootaxa, pp. 141–148.
Schatz, H., 2004. Diversity and global distribution of oribatid mites (Acari, Oribatida): evaluation of the present state of knowledge. Phytophaga 14, 485–500.
Schneider, K., Maraun, M., 2005. Feeding preferences among dark pigmented fungal taxa ("Dematiacea") indicate limited trophic niche differentiation of oribatid mites (Oribatida, Acari). Pedobiologia 49, 61–67.
Schneider, K., Migge, S., Norton, R.A., Scheu, S., Langel, R., Reineking, A., Maraun, M., 2004a. Trophic niche differentiation in soil microarthropods (Oribatida, Acari): evidence from stable isotope ratios ($^{15}N/^{14}N$). Soil Biol. Biochem. 36, 1769–1774.
Schneider, K., Renker, C., Scheu, S., Maraun, M., 2004b. Feeding biology of oribatid mites: a minireview. Phytophaga 14, 247–256.
Schneider, K., Renker, C., Maraun, M., 2005. Oribatid mite (Acari, Oribatida) feeding on ectomycorrhizal fungi. Mycorrhiza 16, 67–72.
Schuster, R., 1956. Der Anteil der Oribatiden an den Zersetzungsvorgängen im Boden. Zeitschrift für Morphologie und Ökologie der Tiere 45, 1–33.
Shimano, S., Sakata, T., Mizutani, Y., Kuwahara, Y., Aoki, J.-i., 2002. Geranial: the alarm pheromone in the nymphal stage of the oribatid mite, *Nothrus palustris*. J. Chem. Ecol. 28, 1831–1837.
Siepel, H., de Ruiter-Dijkman, E.M., 1993. Feeding guilds of oribatid mites based on their carbohydrase activities. Soil Biol. Biochem. 25, 1491–1497.
Siepel, H., 1996. Biodiversity of soil microarthropods: the filtering of species. Biodivers. Conserv. 5, 251–260.
Stötefeld, L., Scheu, S., Rohlfs, M., 2012. Fungal chemical defence alters density-dependent foraging behaviour and success in a fungivorous soil arthropod. Ecol. Entomol. 37, 323–329.
Staaden, S., Milcu, A., Rohlfs, M., Scheu, S., 2011. Olfactory cues associated with fungal grazing intensity and secondary metabolite pathway modulate Collembola foraging behaviour. Soil Biol. Biochem. 43, 1411–1416.
Thomas, R.S., Glen, D.M., Symondson, W.O.C., 2008. Prey detection through olfaction by the soil-dwelling larvae of the carabid predator *Pterostichus melanarius*. Soil Biol. Biochem. 40, 207–216.
Tserng, K.Y., Griffin, R., 2003. Quantitation and molecular species determination of diacylglycerols, phosphatidylcholines, ceramides, and sphingomyelins with gas chromatography. Anal. Biochem. 323, 84–93.
Van den Dool, H., Kratz, P.D., 1963. A generalization of retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. 11, 463–468.
Vegter, J.J., Joosse, E.N.G., Ernsting, G., 1988. Community structure, distribution and population-dynamics of Entomobryidae (Collembola). J. Anim. Ecol. 57, 971–981.
Walter, D.E., 1987. Trophic behavior of mycophagous microarthropods. Ecology 68, 226–229.
Warcup, J.H., 1950. The soil-plate method for isolation of fungi from soil. Nature 166, 117–118.
Wehner, K., Norton, R.A., Blüthgen, N., Heethoff, M., 2016. Specialization of oribatid mites to forest microhabitats—the enigmatic role of litter. Ecosphere 7, e01336.
Wu, C., Jordan, M.D., Newcomb, R.D., Gemmell, N.J., Bank, S., Meusemann, K., Dearden, P.K., Duncan, E.J., Grosser, S., Rutherford, K., Gardner, P.P., Crowhurst, R.N., Steinwender, B., Tooman, L.K., Stevens, M.I., Buckley, T.R., 2017. Analysis of the genome of the New Zealand giant collembolan (*Holacanthella duospinosa*) sheds light on hexapod evolution. BMC Genomics 18, 795.
Zirbes, L., Mescher, M., Vrancken, V., Wathelet, J.P., Verheggen, F.J., Thonart, P., Haubruge, E., 2011. Earthworms use odor cues to locate and feed on microorganisms in soil. PLoS One 6, e21927.
van Berkum, P., 1990. Evidence for a third uptake hydrogenase phenotype among the soybean Bradyrhizobia. Appl. Environ. Microbiol. 56, 3835–3841.

10.4.2 Publication 8: **Imprinted or innate food preferences in the model mite *Archegozetes longisetosus* (Actinotrichida, Oribatida, Trhypochthoniidae)**

Adrian Brückner, Romina Schuster, Timo Smit, and Michael Heethoff

Published in: Soil Organisms 90: 23-26

Submitted: 27.11.2017, Accepted: 21.02.2018, Published: 01.04.2018

Authors contributions:

MH and AB designed the research; RS, TS and AB performed bioassays; AB analyzed the data; AB and MH wrote the paper. All authors read, discussed and approved the final version.

Reprinted by permission from Senckenberg Museum of Natural History Görlitz. Soil Organisms. Imprinted or innate food preferences in the model mite Archegozetes longisetosus (Actinotrichida, Oribatida, Trhypochthoniidae). Brückner Adrian, Schuster Romina, Smit Timo, Heethoff Michael. © Senckenberg Museum of Natural History Görlitz 2018

SOIL ORGANISMS 90 (1) · April 2018 pp. 23–26

SHORT COMMUNICATION

Imprinted or innate food preferences in the model mite *Archegozetes longisetosus* (Actinotrichida, Oribatida, Trhypochthoniidae)

Adrian Brückner*, Romina Schuster, Timo Smit and Michael Heethoff*

Ecological Networks, Technische Univeristät Darmstadt, Schnittspahnstraße 3, 64287 Darmstadt, Germany

* Corresponding authors, e-mail: adrian.brueckner@gmail.com, heethoff@bio.tu-darmstadt.de

Received 27 November 2017 | Accepted 21 February 2018
Published online at www.soil-organisms.de 1 April 2018 | Printed version 15 April 2018

Abstract

Most oribatid mites are opportunistic feeders with a broad variety of different food sources. However, preferences for certain food such as dark pigmented fungi, led to the 'choosy generalist'-hypothesis. The mechanisms behind this idea and whether oribatid mites have an innate or learned preference for food are unknown. We used *Archegozetes longisetosus* Aoki to test whether mites prefer unknown high quality food or food they have experienced before. We found that *A. longisetosus* did not prefer known food, and that food preferences were innate and not due to imprinting/learning behavior.

Keywords Behavioral ecology | soil fauna | choosy generalist

1. Introduction

Oribatid mites (Actinotrichida, Oribatida) feed on a wide range of different resources (Schuster 1956, Luxton 1972, Behan-Pelletier & Hill 1983, Schneider et al. 2004) and show basically a low degree of specialization in other aspects such as microhabitat preferences (Maraun & Scheu 2000, Valdecasas et al. 2006, Wehner et al. 2016). The preference for certain food has been studied in oribatid mites by gut boli/fecal pellet analysis (Anderson 1975, Labandeira et al. 1997, Meier et al. 2002), enzymology (Siepel & de Ruiter-Dijkman 1993, Hubert et al. 2001) or feeding preference tests in the laboratory (Riha 1951, Pande & Berthet 1973, Maraun et al. 1998, Hubert & Lukesova 2001, Maraun et al. 2003, Schneider & Maraun 2005, Koukol et al. 2009). These studies have demonstrated a broad food spectrum for oribatid mites, including leaf-litter, algae, fungi, lichen and dead animals (e.g. collembolans and nematodes). Especially certain types of dark pigmented fungi (family: Dematiaceae Fr.) are readily eaten by oribatids in biotests, yet the reasons for this behavior still remain conspicuous (Maraun et al. 1998, Maraun et al. 2003, Schneider et al. 2004, Schneider & Maraun 2005). In summary, oribatid mites seem to be mostly opportunistic feeders 'choosy generalists'; (Schneider & Maraun 2005), but with preferences, possibly for more nutrient-rich or less toxic food.

Studies on the spider mite *Tetranychus urticae* Koch (Actinotrichida, Prostigmata) and the predatory mite *Neoseiulus californicus* McGregor (Anactinotrichida, Mesostigmata) demonstrated effects of learned food preferences and foraging behavior, respectively (Egas & Sabelis 2001, Egas et al. 2003, Schausberger & Peneder 2017). For example, *T. urticae* with cucumber as food plant induced strong preferences for cucumber (for tomato mite strains), while exposure to tomato induced a strong aversion for tomato in cucumber mite strains (Egas & Sabelis 2001, Egas et al. 2004). Since cucumber as host plant yielded higher reproductive output (= high quality food), compared to tomato (= low quality food) and since both mite strains learned to prefer cucumber over tomato, learning seemed adaptive (Agrawal et al. 2002, Egas et al. 2003).

© Senckenberg Museum of Natural History Görlitz · 2018
ISSN 1864-6417 (print) · **ISSN** 2509-9523 (digital)

open access

This raises the question whether oribatid mites have an innate or imprinted food preference; i.e. do they exhibit an inborn preference for certain resources, which does not change *via* experience of higher quality food (= no learning effects/innate) or do they show preferences for higher quality resources in case they have fed on it before (= learning/imprinted). To test this we used *Archegozetes longisetosus* Aoki (Oribatida, Trhypochthoniidae) – a well-known model species for soil ecology and cell/developmental biology (Heethoff et al. 2007, Heethoff et al. 2013, Heethoff & Scheu 2016, Brückner et al. 2017) – raised on four resources of differing nutritional quality, ultimately resulting in variable reproductive output (for more details see Brückner et al. 2018b).

2. Materials and methods

Archegozetes longisetosus ran (Heethoff et al. 2007) stock cultures were kept in polypropylene boxes grounded with a mixture of plaster of Paris: activated charcoal (9:1) at 28°C and approximately 85–90 % relative humidity in complete darkness. *Archegozetes longisetosus* cultures were fed *ad libitum* with either *Chlorella* powder (Naturya, Bath, UK), lupine flour (Govinda Natur GmbH, Neuhofen, Germany), wheat grass powder (wheat; Naturya, Bath, UK) or dry yeast (Rapunzel Naturkost GmbH, Legau, Germany) three times a week. We offered *Chlorella* powder, lupine flour, wheat grass powder and grinded dry yeast to *A. longisetosu*s which have been cultured on one of the four resources (= origin) for at three generations ($n_{Chlorella}$= 120 specimens; n_{lupine}= 590 specimens; n_{wheat}= 300 specimens; n_{yeast}= 300 specimens; different numbers are due to different breeding successes over the three generations), to test whether oribatid mites are imprinted to a resource they experienced as juvenile instars or possess an innate preference for certain food. The experiments were performed in plastic petri-dishes (4.5 × 1.5 cm) grounded with moist analytical filter paper (both, Hartenstein GmbH, Würzburg, Germany), and the food powders were placed marginally in a circle to guarantee maximum distance. Ten specimens were used for every replicate (n = 131). After one hour we counted the number of specimens resting on each of the offered resources. Unresponsive individuals were not counted and excluded from the analysis. We used a generalized mixed effect model (GLMM) with overall oribatid mite counts as response variable, origin and resource choice as fixed factors and experimental ID as random factor. The GLMM was fitted with a negative-binomial error distribution (goodness-of-fit test for the response variable: χ^2 = 6.9; p = 0.44) and log as link-function. Overall preference differences among the food sources were accessed using Wilcoxon signed-rank tests with affiliated false-discovery rate correction (Benjamini & Hochberg 1995). Statistics were performed in R 3.3.1 'Bug in Your Hair' (R Core Team 2016) using the R packages 'nlme' (Pinheiro et al. 2017), 'lme4' (Bates et al. 2015) and 'car' (Fox & Weisberg 2011).

3. Results

Archegozetes longisetosus preferred distinct resources (resource choice: Wald-χ2 = 9.6; df = 3; P = 0.022; Tab. 1), but origin had no effect (origin: Wald-χ2 = 4.36; df = 3; P = 0.215). Accordingly, preferences were not influenced by the resources the specimens developed on (interaction resource choice x origin: Wald-χ2 = 2.84; df = 9; P = 0.971). Overall, *A. longisetosus* individuals were mostly attracted by lupine, to a lesser extent by wheat and yeast, and least by *Chlorella* (see pairwise tests in Tab. 1).

4. Discussion

Archegozetes longisetosus did not prefer well-known resources, but rather showed an innate general preference (Tab. 1). Interestingly, this innate behaviour was not related to reproductive fitness, because the most preferred resource, lupine (number off offspring per female: 18.98 ± 4.48; mean ± SD), does not generate the highest number of offspring compared to the other three resource (chlorella: 1.44 ± 0.56; wheat: 44.76 ± 5.41; yeast: 22.20 ± 2.01), but rather represents a food with intermediate offspring production (numbers are extracted from Brückner et al. 2018b). The lack of any imprinting was rather surprising, because the used mite stock-cultures

Table 1. Proportions of *Archegozetes longisetosus* individuals counted on the different food sources depending on their origin and the total mean proportion (± SD) of mites on each chosen resource. The same letters indicate no differences among groups (P > 0.05) in pairwise Wilcoxon test comparisons of the overall resource choice after false discovery rate correction.

origin	**resource choice**			
	chlorella	lupine	wheat	yeast
chlorella	20	44	19	17
lupine	14	35	25	26
wheat	17	25	28	30
yeast	17	36	25	22
total	**17 ± 2 a**	**35 ± 7 c**	**24 ± 3 b**	**24 ± 5 b**

had exclusively been raised on one of the four offered resources for several generations before the experiment. Based on the learning effect found in *T. urticae* (e.g. Egas et al. 2003, Egas et al. 2004) we had expected to find at least a certain imprinted preference of wheat/yeast raised mite for their resource or a general switch to the resource which results in the highest reproductive output. This was, however, not the case and the strong innate preference for lupine may thus be caused by other than fitness related attributes (e.g., olfactory signals). Indeed, lupine powder was the food with the highest fatty acid content (Brückner et al. 2017) of all offered resource, supporting the idea that fatty acids could serve as important olfactory signals in *A. longisetosus* (see Brückner et al. 2018a).

Furthermore, food preference imprinting may be not beneficial for a highly opportunistic/generalist oribatid mite species like *A. longisetosus*, since such species need to switch food resources quite regularly to obtain exploitable nutrients in an environment with patchy distributed resources (Farley & Fitter 1999, Hodge 2006), and high densities of potential competitors (Hassall et al. 1986). Additionally, compared to plant parasites and predators (Egas & Sabelis 2001, Egas et al. 2003, Schausberger & Peneder 2017), learned associations may not be necessary for detritivores (see also Sitvarin et al. 2015), since they actually 'live' in their own food substrate and thus do not need to forage for distant food sources, a process often related to higher energetic costs (e.g. Schowalter 2016).

5. Acknowledgments

AB was supported by a PhD scholarship of the German Nation Academic Foundation (Studienstiftung des deutschen Volkes). This study was funded by the German Science Foundation (DFG; HE 4593/5-1).

6. Authors' contributions

MH and AB designed the research; RS, TS and AB performed bioassays; AB analysed the data; AB and MH wrote the paper. All authors read, discussed and approved the final version.

7. Competing financial interests

The authors declare no competing financial interests.

8. References

Agrawal, A. A., F. Vala & M. W. Sabelis (2002): Induction of preference and performance after acclimation to novel hosts in a phytophagous spider mite: adaptive plasticity? – American Naturalist **159**: 553–565.

Anderson, J. M. (1975): Succession, diversity and trophic relationships of some soil animals in decomposing leaf litter. – Journal of Animal Ecology **44**: 475–495.

Bates, D., M. Maechler, B. Bolker & S. Walker (2015): Fitting linear mixed-effects models using lme4. – Journal of Statistical Software **67**: 1–48.

Behan-Pelletier, V. M. & S. B. Hill (1983): Feeding-habits of sixteen species of Oribatei (Acari) from an acid peat bog, Glenamoy, Ireland. – Revue D'écologie et de Biologie du Sol **20**: 221–267.

Benjamini, Y. & Y. Hochberg (1995): Controlling the false discovery rate - a practical and powerful approach to multiple testing. – Journal of the Royal Statistical Society Series B-Methodological **57**: 289–300.

Brückner, A., A. Hilpert & M. Heethoff (2017): Biomarker function and nutritional stoichiometry of neutral lipid fatty acids and amino acids in oribatid mites. – Soil Biology & Biochemistry **115**: 35–43.

Brückner, A., R. Schuster, T. Smit, M. M. Pollierer, I. Schäffler & M. Heethoff (2018a): Track the snack – Olfactory cues shape foraging behaviour of decomposing soil mites (Oribatida). – Pedobiologia **66**: 74–80.

Brückner, A., R. Schuster, K. Wehner & M. Heethoff (2018b) Adding to the reproductive biology of *Archegozetes longisetosus* (Actinotrichida, Oribatida, Trhypochthoniidae) again: the role of nutritional quality. – Soil Organisms **90**(1): **1–12**.

Egas, M. & M. W. Sabelis (2001): Adaptive learning of host preference in a herbivorous arthropod. – Ecology Letters **4**: 190–195.

Egas, M., D. J. Norde & M. W. Sabelis (2003): Adaptive learning in arthropods: spider mites learn to distinguish food quality. – Experimental & Applied Acarology **30**: 233–247.

Egas, M., U. Dieckmann & M. W. Sabelis (2004): Evolution restricts the coexistence of specialists and generalists: the role of trade-off structure. – American Naturalist **163**: 518–531.

Farley, R. A. & A. H. Fitter (1999): The responses of seven co-occurring woodland herbaceous perennials to localized nutrient-rich patches. – Journal of Ecology **87**: 849–859.

Fox, J. & S. Weisberg (2011): Companion to applied regression, Second Edition. – Thousand Oaks CA: Sage [http://socserv.socsci.mcmaster.ca/jfox/Books/Companion].

Hassall, M., S. Visser & D. Parkinson (1986): Vertical migration of *Onychiurus subtenuis* (Collembola) in relation to rainfall and microbial activity. – Pedobiologia **29**: 175–182.

Heethoff, M. & S. Scheu (2016): Reliability of isotopic fractionation (Δ15N, Δ13C) for the delimitation of trophic levels of oribatid mites: Diet strongly affects Δ13C but not Δ15N. – Soil Biology & Biochemistry **101**: 124–129.

Heethoff, M., P. Bergmann, M. Laumann & R. A. Norton (2013): The 20th anniversary of a model mite: A review of current knowledge about *Archegozetes longisetosus* (Acari, Oribatida). – Acarologia **53**: 353–368.

Heethoff, M., M. Laumann & P. Bergmann (2007): Adding to the reproductive biology of the parthenogenetic oribatid mite, *Archegozetes longisetosus* (Acari, Oribatida, Trhypochthoniidae). – Turkish Journal of Zoology **31**: 151–159.

Hodge, A. (2006): Plastic plants and patchy soils. – Journal of Experimental Botany **57**: 401–411.

Hubert, J. & A. Lukesova (2001): Feeding of the panphytophagous oribatid mite *Scheloribates laevigatus* (Acari: Oribatida) on cyanobacterial and algal diets in laboratory experiments. – Applied Soil Ecology **16**: 77–83.

Hubert, J., M. Zilova & S. Pekar (2001): Feeding preferences and gut contents of three panphytophagous oribatid mites (Acari: Oribatida). – European Journal of Soil Biology **37**: 197–208.

Koukol, O., J. Mourek, Z. Janovsky & K. Cerna (2009): Do oribatid mites (Acari: Oribatida) show a higher preference for ubiquitous vs. specialized saprotrophic fungi from pine litter? – Soil Biology & Biochemistry **41**: 1124–1131.

Labandeira, C. C., T. L. Phillips & R. A. Norton (1997): Oribatid mites and the decomposition of plant tissues in Paleozoic coal-swamp forests. – Palaios **12**: 319–353.

Luxton, M. (1972): Studies on oribatid mites of a Danish beech wood soil .1. Nutritional biology. – Pedobiologia **12**: 434–463.

Maraun, M. & S. Scheu (2000): The structure of oribatid mite communities (Acari, Oribatida): Patterns, mechanisms and implications for future research. – Ecography **23**: 374–383.

Maraun, M., S. Migge, M. Schaefer & S. Scheu (1998): Selection of microfungal food by six oribatid mite species (Oribatida, Acari) from two different beech forests. – Pedobiologia **42**: 232–240.

Maraun, M., H. Martens, S. Migge, A. Theenhaus & S. Scheu (2003): Adding to 'the enigma of soil animal diversity': fungal feeders and saprophagous soil invertebrates prefer similar food substrates. – European Journal of Soil Biology **39**: 85–95.

Meier, F. A., S. Scherrer & R. Honegger (2002): Faecal pellets of lichenivorous mites contain viable cells of the lichen-forming ascomycete *Xanthoria parietina* and its green algal photobiont, *Trebouxia arboricola*. – Biological Journal of the Linnean Society **76**: 259–268.

Pande, Y. D. & P. Berthet (1973): Studies on the food and feeding habits of soil Oribatei in a black pine plantation. – Oecologia, **12**: 413–426.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar & R. C. Team (2017): nlme: Linear and nonlinear mixed effects models. – R package version 3.1-131 [https://CRAN.R-project.org/package=nlme].

R Core Team (2016): R: A language and environment for statistical computing. R Foundation for Statistical Computing, – Vienna, Austria [http://www.R-project.org].

Riha, G. (1951): Zur Ökologie der Oribatiden in Kalksteinböden. Zoologische Jahrbücher **80**: 407–450.

Schausberger, P. & S. Peneder (2017): Non-associative versus associative learning by foraging predatory mites. – BMC Ecology **17**: 2.

Schneider, K. & M. Maraun (2005): Feeding preferences among dark pigmented fungal taxa ("Dematiacea") indicate limited trophic niche differentiation of oribatid mites (Oribatida, Acari). – Pedobiologia **49**: 61–67.

Schneider, K., C. Renker, S. Scheu & M. Maraun (2004): Feeding biology of oribatid mites: a minireview. – Phytophaga **14**: 247–256.

Schowalter, T. D. (2016): Insect Ecology: An Ecosystem Approach. – Academic Press, New York.

Schuster, R. (1956): Der Anteil der Oribatiden an den Zersetzungsvorgängen im Boden. – Zeitschrift für Morphologie und Ökologie der Tiere **45**: 1–33.

Siepel, H. & E. M. de Ruiter-Dijkman (1993): Feeding guilds of oribatid mites based on their carbohydrase activities. – Soil Biology & Biochemistry **25**: 1491–1497.

Sitvarin, M. I., C. Romanchek & A. L. Rypstra (2015): Nonconsumptive predator-prey interactions: sensitivity of the detritivore *Sinella curviseta* (Collembola: Entomobryidae) to cues of predation risk from the spider *Pardosa milvina* (Araneae: Lycosidae). – Environmental Entomology **44**: 349–355.

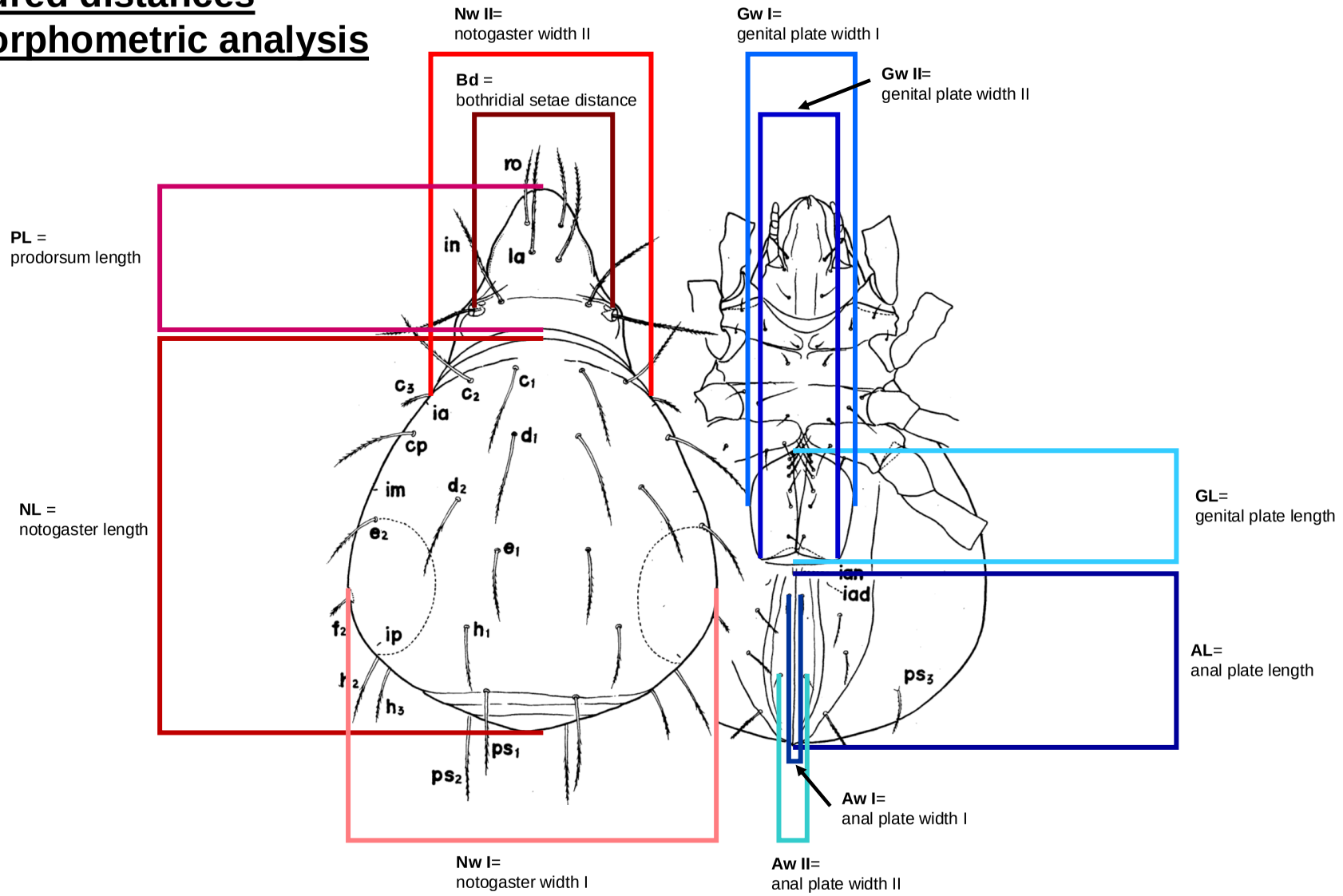
Valdecasas, A. G., A. I. Camacho & M. L. Pelaez (2006): Do small animals have a biogeography? – Experimental & Applied Acarology **40**: 133–144.

Wehner, K., R. A. Norton, N. Blüthgen & M. Heethoff (2016): Specialization of oribatid mites to forest microhabitats—the enigmatic role of litter. – Ecosphere **7**: e01336.

10.5 Appendix

10.5.1 Supplementary information - Publication 2

Measured distances for morphometric analysis



Modified after:
Aoki J. (1965). Oribatiden (Acarina) Thailands I. Nature and Life in Southeast Asia, 4: 129-193.

Secretion amount [ng/µg] Dunn's posthoc test (false discovery rate corrected):

	blood	chlor	hemp	yeast	bone	lupine	fungi	pollen	spiru
chlor	0.00417	-	-	-	-	-	-	-	-
hemp	0.26029	0.08162	-	-	-	-	-	-	-
yeast	0.00011	0.42059	0.00796	-	-	-	-	-	-
bone	0.15316	0.15934	0.70570	0.01952	-	-	-	-	-
lupine	0.00017	0.44397	0.00985	0.97461	0.02497	-	-	-	-
fungi	2.0e-08	0.01439	1.3e-05	0.09656	4.8e-05	0.09685	-	-	-
pollen	0.06711	0.29261	0.46562	0.05871	0.69711	0.06711	0.00021	-	-
spiru	4.8e-05	0.25366	0.00355	0.69711	0.00985	0.69711	0.21962	0.02530	-
wheat	0.01526	0.62566	0.19797	0.17894	0.34467	0.19797	0.00250	0.59318	0.09656

Regeneration [%]Dunn's posthoc test (false discovery rate corrected):

	blood	chlor	hemp	yeast	bone	lupine	fungi	pollen	spiru
chlor	0.05669	-	-	-	-	-	-	-	-
hemp	1.6e-05	0.04771	-	-	-	-	-	-	-
yeast	9.8e-05	0.11440	0.76493	-	-	-	-	-	-
bone	0.00286	0.44371	0.31468	0.50775	-	-	-	-	-
lupine	0.00097	0.31468	0.44371	0.70767	0.83992	-	-	-	-
fungi	0.04771	0.92359	0.05634	0.14354	0.49991	0.37591	-	-	-
pollen	0.00058	0.27677	0.50665	0.77142	0.76493	0.90612	0.31468	-	-
spiru	1.6e-05	0.04609	0.92800	0.70767	0.29719	0.41863	0.04771	0.47283	-
wheat	4.8e-05	0.07756	0.83992	0.89471	0.44371	0.57254	0.09871	0.69443	0.80461

Body mass [µg] Dunn's posthoc test (false discovery rate corrected):

	blood	chlor	hemp	yeast	bone	lupine	fungi	pollen	spiru
chlor	2.9e-10	-	-	-	-	-	-	-	-
hemp	2.9e-05	0.01150	-	-	-	-	-	-	-
yeast	1.2e-08	0.37429	0.07900	-	-	-	-	-	-
bone	0.00046	0.00096	0.47747	0.01385	-	-	-	-	-
lupine	0.00046	0.00096	0.47747	0.01385	0.98999	-	-	-	-
fungi	0.01385	1.9e-05	0.07093	0.00046	0.30210	0.30210	-	-	-
pollen	0.07093	3.1e-08	0.00486	1.9e-06	0.03977	0.03977	0.40469	-	-
spiru	0.00014	0.03004	0.95885	0.15396	0.47747	0.47747	0.09574	0.01245	-
wheat	0.03977	1.4e-07	0.01150	8.0e-06	0.07093	0.07093	0.52979	0.79407	0.02216

Developmental time [days] Dunn's posthoc test (false discovery rate corrected):

	chlor	hemp	yeast	bone	lupine	fungi	pollen	spiru
hemp	0.09575	-	-	-	-	-	-	-
yeast	0.02883	0.55384	-	-	-	-	-	-
bone	0.00848	2.2e-07	2.7e-09	-	-	-	-	-
lupine	0.04661	9.9e-06	1.6e-07	0.37011	-	-	-	-
fungi	0.00041	1.5e-09	1.1e-11	0.29632	0.04661	-	-	-
pollen	0.55675	0.00839	0.00069	0.01212	0.08998	0.00041	-	-
spiru	0.00870	8.6e-05	1.7e-05	0.31838	0.13022	0.70515	0.01505	-
wheat	0.31251	0.00094	5.1e-05	0.04450	0.25582	0.00212	0.55675	0.03282

Survival [%] Dunn's posthoc test (false discovery rate corrected):

	blood	chlor	hemp	yeast	bone	lupine	fungi	pollen	spiru
chlor	0.77731	-	-	-	-	-	-	-	-
hemp	0.00018	3.5e-05	-	-	-	-	-	-	-
yeast	0.00016	2.9e-05	0.98373	-	-	-	-	-	-
bone	0.00670	0.00383	0.23212	0.22463	-	-	-	-	-
lupine	9.8e-07	2.7e-08	0.15476	0.15476	0.00669	-	-	-	-
fungi	0.35988	0.48650	0.00060	0.00051	0.03165	1.1e-06	-	-	-
pollen	0.00013	2.0e-05	0.92596	0.92596	0.18691	0.19758	0.00035	-	-
spiru	0.77731	0.95626	0.00122	0.00111	0.02752	1.5e-05	0.65809	0.00083	-
wheast	5.6e-09	2.1e-11	0.01063	0.01063	0.00015	0.35988	2.1e-09	0.01671	1.6e-07

Total offspring [N*female^{-1}] Dunn's posthoc test (false discovery rate corrected):

	blood	chlor	hemp	yeast	bone	lupine	fungi	pollen	spiru
chlor	0.30727	-	-	-	-	-	-	-	-
hemp	9.1e-05	0.00033	-	-	-	-	-	-	-
yeast	7.2e-09	6.6e-09	0.02925	-	-	-	-	-	-
Bone	0.00570	0.03196	0.16583	0.00027	-	-	-	-	-
lupine	5.3e-07	1.2e-06	0.18735	0.42985	0.00709	-	-	-	-
fungi	0.14257	0.57375	0.00270	1.1e-07	0.12768	1.6e-05	-	-	-
pollen	0.00012	0.00050	0.90848	0.02237	0.19530	0.15954	0.00384	-	-
spiru	0.30727	0.86763	0.00847	1.1e-05	0.13936	0.00023	0.80214	0.01079	-
wheat	1.7e-12	2.1e-13	0.00019	0.13729	2.1e-07	0.02237	4.7e-12	0.00012	1.5e-08

Reproductive output [mg*female^{-1}] Dunn's posthoc test (false discovery rate corrected):

	blood	chlor	hemp	yeast	bone	lupine	fungi	pollen	spiru
chlor	0.08950	-	-	-	-	-	-	-	-
hemp	2.7e-06	0.00053	-	-	-	-	-	-	-
yeast	1.6e-08	4.9e-06	0.30856	-	-	-	-	-	-
bone	0.00053	0.04615	0.15886	0.01367	-	-	-	-	-
lupine	1.8e-09	6.0e-07	0.11082	0.56299	0.00291	-	-	-	-
fungi	0.09792	0.98146	0.00068	7.7e-06	0.04688	9.8e-07	-	-	-
pollen	7.8e-05	0.01153	0.32510	0.04289	0.65668	0.01005	0.01291	-	-
spiru	0.15886	0.87473	0.00102	2.0e-05	0.04688	3.0e-06	0.87497	0.01367	-
wheat	2.0e-12	4.5e-10	0.00546	0.07453	2.0e-05	0.26438	8.8e-10	9.3e-05	6.5e-09

10.5.2 Supplementary information - Publication 4

Supplementary information to:

Nutritional effects on chemical defense alter predator-prey dynamics

Adrian Brückner[1] and Michael Heethoff[1]

[1]*Ecological Networks, Technische Universität Darmstadt, Schnittspahnstraße 3, 64287 Darmstadt, Germany*

**Corresponding authors: adrian.brueckner@gmail.com; heethoff@bio.tu-darmstadt.de*

S1 - Experimental setup – resource powders and nutrient analysis

Ten different diets of several types were offered *ad libitum* to *Archegozetes longisetosus*: animal resources such as blood meal (blood; Common Baits, Rosenfeld, Germany) and bone meal (bone; Canina Pharma GmbH, Hamm, Germany); one bacterial resource, Spirulina powder (spirulina; Interaquaristik, Biedenkopf-Breidenstein, Germany); fungal resources, such as shiitake fungus powder (fungi; Arche Naturprodukte GmbH, Hilden, Germany) and grinded dry yeast (yeast; Rapunzel Naturkost GmbH, Legau, Germany); and a variety of herbal resources, such as Chlorella powder (chlorella; Naturya, Bath, UK), hemp protein powder (hemp; Naturya, Bath, UK), sweet lupine flour (lupine; Govinda Natur GmbH, Neuhofen, Germany), grinded mixed pollen grain (pollen; Ascopharm GmbH, Wernigerode, Germany) and wheat grass powder (wheat; Naturya, Bath, UK).

Fatty acid analysis (based on Brückner et al. 2017 and Frank et al. 2017)

Total neutral lipids (hereafter, neutral lipid fatty acids = NLFAs) of the resource poweders were extracted from using 1 ml of a chloroform:methanol-mixture, 2:1 (V/V) over a period of 24 h. Afterwards extracts were purified and separated; briefly: SiOH-columns (Chromabond® SiOH, Macherey-Nagel GmbH & Co. KG, Düren, Germany) were washed and conditioned with 6 ml hexane. Afterwards samples were applied on the column and elution of neutral lipids was accomplished with 4 ml of chloroform. Afterwards the chloroform fraction was evaporated to dryness under nitrogen gas flow and residuals were redissolved in dichloromethane:methanol, 2:1 (V/V). 20 µl were transferred to chromatographic glass vials with a conical inlet (150 µl), 20 µl nonadecanoic acid (220 ng/µl) was additionally added as internal standard and the mixture was evaporated to dryness again and subsequently derivatized to fatty acid methyl esters (FAMEs) with TMSH (trimethylsulfonium hydroxide; 0.25 M in MeOH from Fluka, Sigma-Aldrich, St. Louis, USA) reagent according to the supplier's information.Samples were analysed with a QP2010 Ultra GC/MS (Shimadzu, Duisburg, Germany). The gas chromatograph (GC) was equipped with a ZB-5MS fused silica capillary column (30 m x 0.25 mm ID, df= 0.25 µm) from Phenomenex (Aschaffenburg, Germany). 1 µl sample aliquots were injected by using an AOC-20i autosampler-system (Shimadzu, Duisburg, Germany) into a PTV-split/splitless-injector (Optic 4, ATAS GL, Eindhoven, Netherlands), which operated in splitless-mode. Injection-temperature was programmed from initial 70 °C up to 300 °C and then an isothermal hold for 59 minutes. Hydrogen was used as carrier-gas with a constant flow rate of 2.89 ml/min. The temperature of the GC oven was raised from initial 60°C for 1 min, to

150°C with a heating-rate of 15°C/min, to 260°C with a heating-rate of 3°C/min, to 320°C with a heating-rate of 10°C/min and then an isothermal hold at 320°C for 10 min. Electron ionization mass spectra were recorded at 70 eV from m/z 40 to 650. The ion source of the mass spectrometer and the transfer line were kept at 250°C. FAMEs were identified by comparison with the FAME and BAME analytical standards (Sigma-Aldrich, St. Lois, USA). The configurations of the double bonds were not specifically determined.

Amino acid analysis (based on Brückner et al. 2017 and Frank et al. 2017)

For analysis of the amino acids, 5 mg (± 0.1 mg) dried poweders was diluted in 200 µL of hydrochloric acid (6 mol/l) and boiled for four hours at 100°C, and cooled to room temperature afterwards. Note that this acidic chemical extraction decays asparagine, glutamine and tryptophan. Afterwards residuals were cooled to room temperature, centrifuged (10 min at 14,800 rpm) and the supernatants were transferred into fresh tubes and were evaporated to dryness at 100°C, before the samples were re-dissolved in 200 µL of deionized water and evaporated repeatedly. Samples subsequently were re-dissolved again in 200 µL of deionized water and and measured with an ion exchange chromatograph with ninhydrin post-column derivatization (Biochrom 20+, Amino Acid Analyzer, Cambridge, UK). A standard amino acid mixture (Laborservice Onken GmbH, Gründau, Germany) was used as external standard. C/N analysis

C/N analysis (based on Brückner et al. 2017 and Frank et al. 2017)

Dried poweder samples were mixed with hydrochloric acid (HCl; approx. 0.05 mol/l) to remove potential inorganic carbonate as CO_2. Samples were subsequently dried again and weighed into tin capsules (6±1 mg dry weight). Total organic carbon and nitrogen contents were measured by an elemental analyzer (EA 1108 Elemental Analyser, Carlo Erba, Milan, Italy). Acetanilide (Merck, Darmstadt, Germany) was used for internal calibration.

References:

Brückner A., Hilpert A., Heethoff M. (2017) Biomarker function and nutritional stoichiometry of neutral lipid fatty acids and amino acids in oribatid mites. Soil Biol Biochem 115: 35-43.

Frank K., Brückner A., Hilpert A., Heethoff M., Blüthgen N. (2017) Nutrient quality of vertebrate dung as a diet for dung beetles. Sci Rep 7:12141.

Tab S2 Nutrient analyses of the ten resources

Resource	Fat, i.e. NLFAs [µg/mg]	Protein, i.e. amino acids [µg/mg]	Carbohydrates [µg/mg]	C/N ratio
blood	3.0	622.2	0	3.3
bone	90.8	336.9	50	4.2
chlorella	30.4	313.1	70	5.4
fungi	19.0	102.1	250	11.3
hemp	121.7	281.1	260	5.6
lupine	169.0	261.3	100	7.0
pollen	33.0	95.0	670	13.6
spirulina	42.3	394.7	250	4.3
wheat	13.1	85.9	220	12.4
yeast	23.4	193.9	110	7.5

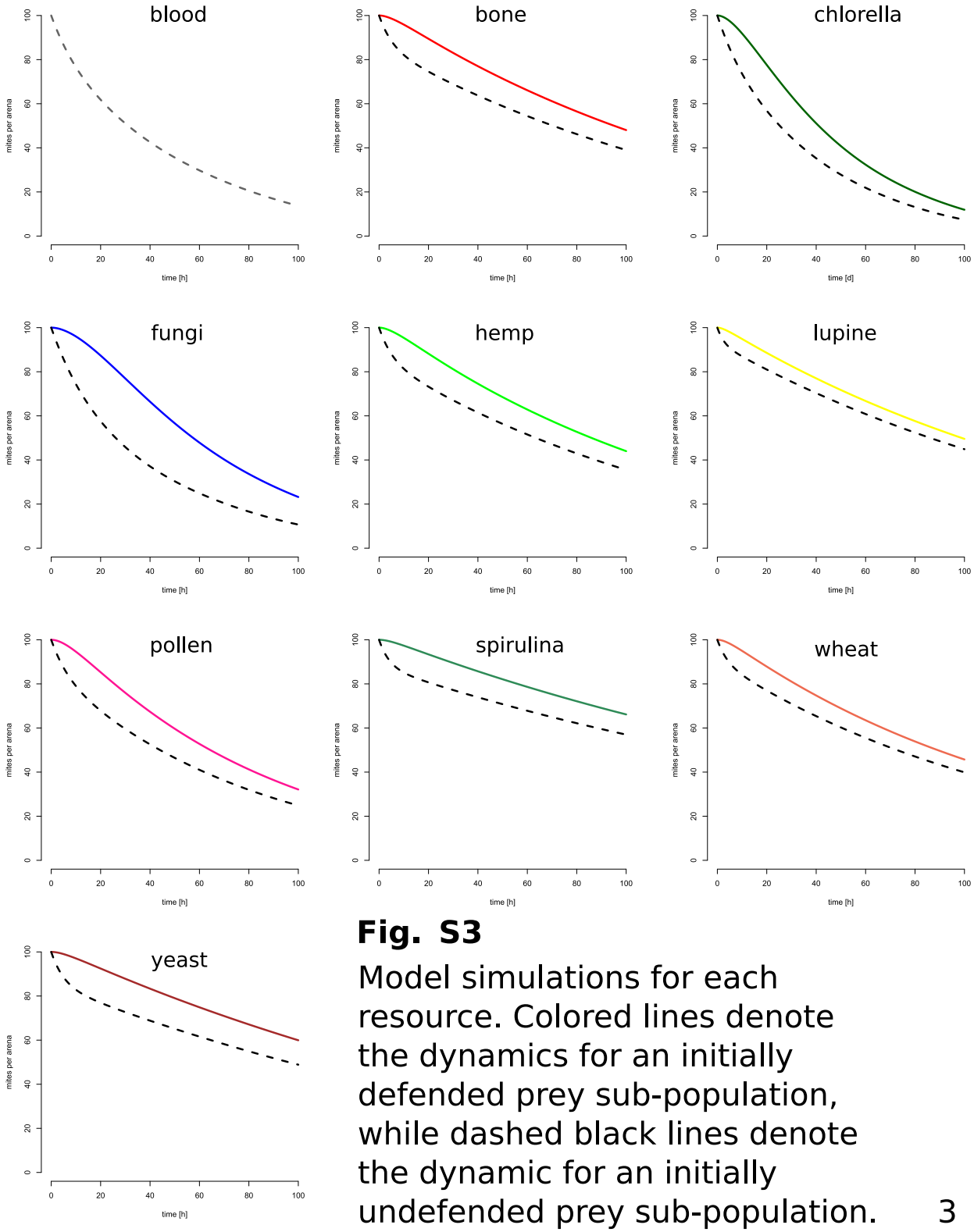


Fig. S3

Model simulations for each resource. Colored lines denote the dynamics for an initially defended prey sub-population, while dashed black lines denote the dynamic for an initially undefended prey sub-population.

10.5.3 Supplementary information - Publication 6

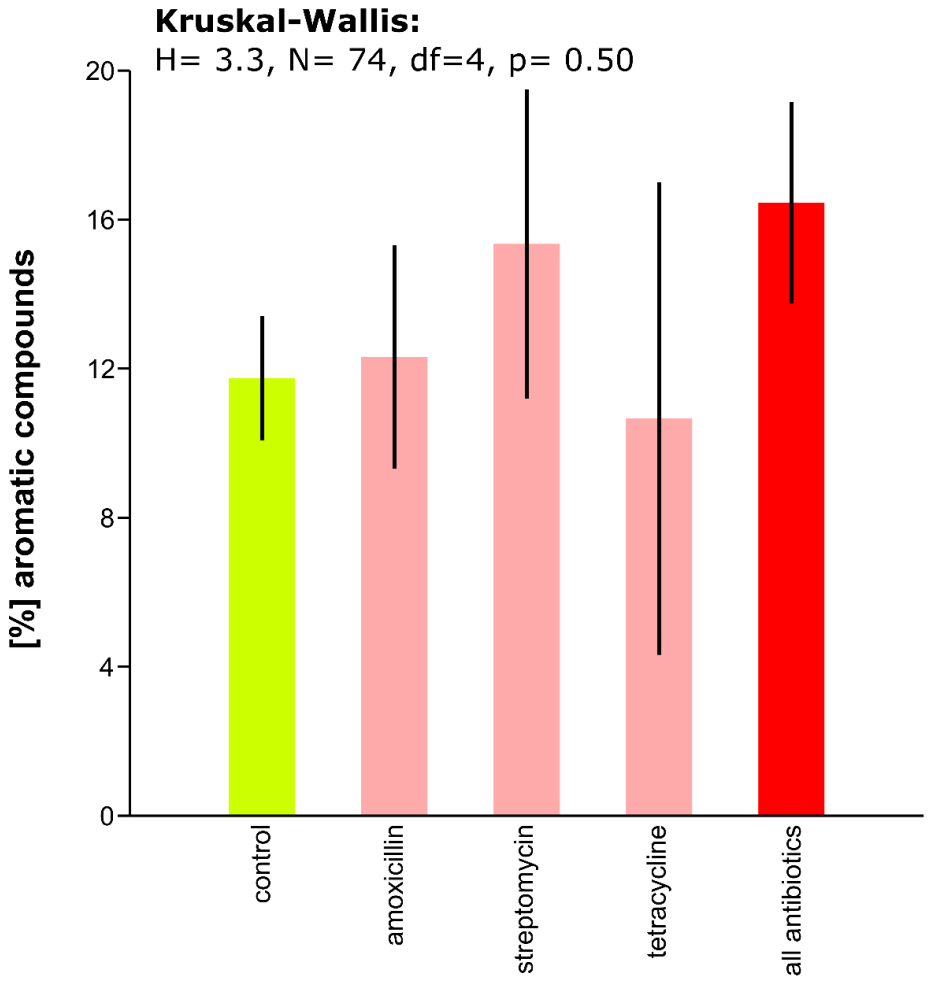


Fig. S1. The effect of different antibiotic treatments - amoxicillin, streptomycin, tetracycline, and a combination of all antibiotics - on the relative aromatic compound ion abundances (%; calculated from ion abundances) compared to the control group. Colored bars represent means, error indicators are standard errors. In-figure text denotes the result and statistical parameters of a non-significant Kruskal-Wallis test. df= degrees of freedom; N= sample size.

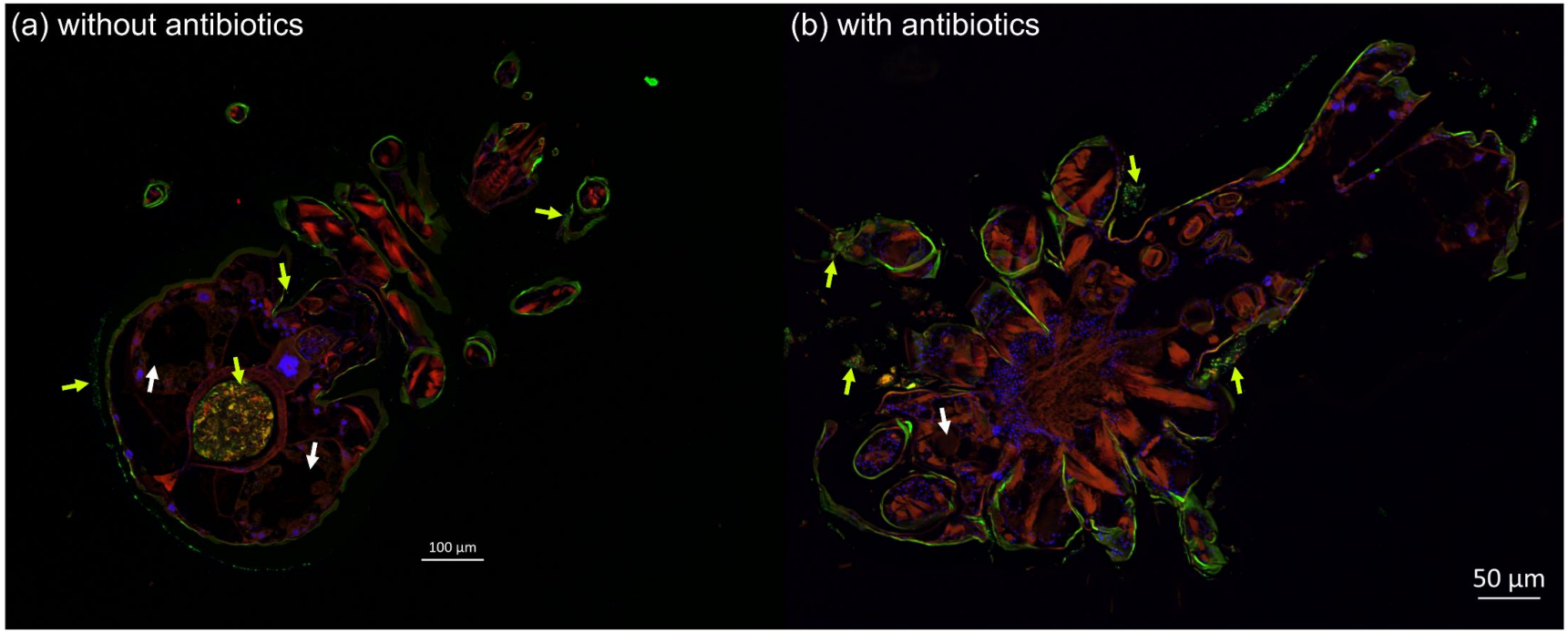


Fig. S2. Fluorescence *in situ* hybridization (FISH) focusing on the cuticular surface of *Archegozetes longisetosus*. The left side of the figure (a) shows a frontal section of a mite fed with untreated wheat-grass powder. Bacterial signals (green arrow heads) were recovered on the cuticle (see four example arrows) and also on/within a fecal pellet in the alimentary tract. Yet, no signals were detected in the caecae (white arrow heads). The right side (b) shows another frontal plane (more ventrally), from a mite treated with a mixture of antibiotics (10% w/w; combined amoxicillin, streptomycin and tetracycline; oral in the diet). No bacterial signals were recovered from the alimentary tract (white arrow head). However, signals were still detected on the cuticle (green arrow heads). Bacteria stained in green with the general bacterial probe EUB338-Cy5; note the different scale bars.

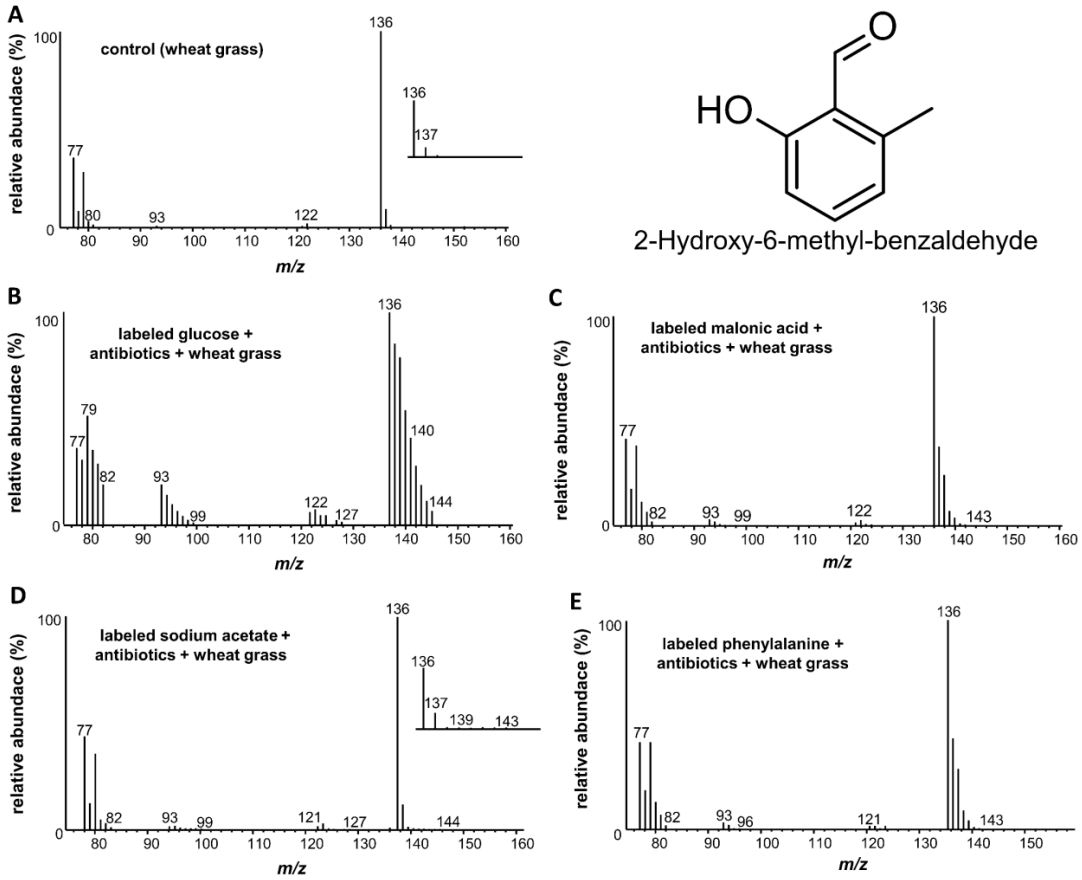


Fig. S3. Representative mass spectra of 2-hydroxy-6-methyl-benzaldehyde extracted from mites fed with unlabeled (A), ^{13}C/d-labeled glucose (B), ^{13}C-malonic acid (C), ^{13}C-acetat (D) and ^{13}C-phenylalanine (E) recorder in single-ion mode. Inserts show the $[M+1]^+$-ion series in the control (A) and ^{13}C-acetat (D).

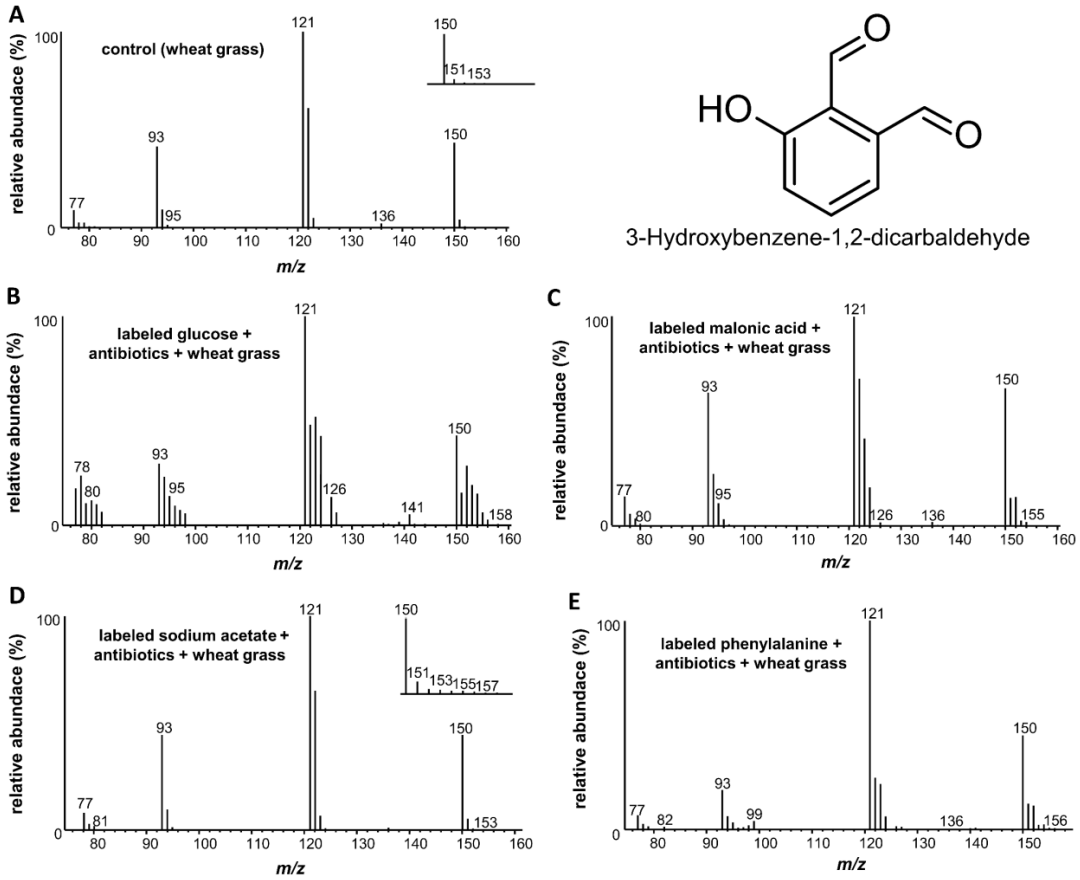


Fig. S4. Representative mass spectra of 3-hydroxybenzene-1,2-dicarbaldehyde extracted from mites fed with unlabeled (A), ^{13}C/d-labeled glucose (B), ^{13}C-malonic acid (C), ^{13}C-acetat (D) and ^{13}C-phenylalanine (E) recorder in single-ion mode. Inserts show the $[M+1]^+$-ion series in the control (A) and ^{13}C-acetat (D).

10.5.4 Supplementary information - Publication 7

substance	time	lichen VOCs (%)	litter VOCs (%)	fungi VOCs (%)	bacteria VOCs (%)	hit (Wiley/NIST 2009) or m/z fragmentation (for unknowns, sorted in order of relative intensity)	RI	lit_RI	RI ref
propanoic acid	6.16	0.00	2.19	7.07	0.01	90	680	679	Pérès, C.; Begnaud, F.; Berdagué, J.-L., Fast characterization of Camembert cheeses by static headspace-mass spectrometry, Sens. Actuators, 2002, 87, 3, 491-497.
pentanal	6.52	3.94	0.61	-	-	93	696	696	Engel, E.; Ratel, J., Correction of the data generated by mass spectrometry analyses of biological tissues: Application to food authentication, J. Chromatogr. A, 2007, 1154, 1-2, 331-341.
unknown	7.41	-	3.35	-	-	m/z= 81, 79, 95, 110 (M+), 41, 43, 67, 53, 55, 77, 54, 65, 73, 91, 109, 51, 80, 57, 66, 82, 42, 68, 83, 40	740	-	-
isobutric acid	7.55	-	-	6.25	0.07	93	742	744	Pino, J.A.; Marquez, E.; Marbot, R., Volatile constituents from tea of roselle (Hibiscus sabdariffa L.), Rev. CENIC Ciencias Quimicas, 2006, 37, 3, 127-129.
1-pentanol	7.93	7.84	0.71	-	-	96	762	764	Cho, I.H.; Namgung, H.-J.; Choi, H.-K.; Kim, Y.-S., Volatiles and key odorants in the pileus and stipe of pine-mushroom (Tricholoma matsutake Sing.), Food Chem., 2008, 106, 1, 71-76.
2-pentene-1-ol	8.02	-	0.10	-	-	88	767	767	Mondello, L., HS-SPME-GCxGC-MS analysis of Yerba Mate (Ilex paraguariensis) in Shimadzu GC-GC application compendium of comprehensive 2D GC, Vol. 1-5, Shimadzu Corp., 2012, 1-29.
butanoic acid	8.10	-	0.38	1.49	0.02	91	774	775	Pérès, C.; Begnaud, F.; Berdagué, J.-L., Fast characterization of Camembert cheeses by static headspace-mass spectrometry, Sens. Actuators, 2002, 87, 3, 491-497.
3-methyl-2-butenal	8.39	-	2.50	0.09	1.02	95	783	782	Nance, M.R.; Setzer, W.N., Volatile components of aroma hops (Humulus lupulus L.) commonly used in beer brewing, J. of Brewing and Distilling, 2011, 2, 2, 16-22.
UNK 794	8.60	-	0.18	-	-	m/z= 71, 41, 43, 45, 44, 42, 55, 85, 45, 60, 56, 100 (M+ ?)	794	-	-
hexanal	8.75	26.26	8.31	-	-	99	800	800	Engel, E.; Ratel, J., Correction of the data generated by mass spectrometry analyses of biological tissues: Application to food authentication, J. Chromatogr. A, 2007, 1154, 1-2, 331-341.
methyl-pyrazine	9.39	-	-	0.27	0.60	92	824	823	Hierro, E.; de la Hoz, L.; Ordóñez, J.A., Headspace volatile compounds from salted and occasionally smoked dried meats

									(cecinas) as affected by animal species, Food Chem., 2004, 85, 4, 649-657.
iso valeric acid	9.52	-	0.11	0.36	0.58	92	829	833	Fokialakis, N.; Magiatis, P.; Mitaku, S., Essential oil constituents of Valeriana italica and Valeriana tuberosa. Stereochemical and conformational study of 15-acetoxyvaleranone, Z. Naturforsch., 2002, 57c, 791-796.
1H-pyrrole, 3-methyl-	9.74	-	0.03	-	-	94	838	841	Pino, J.A.; Marbot, R.; Vazquez, C., Characterization of volatiles in Loquat fruit (Eriobotrya japonica Lindl.), Revista CENIC Ciencias Quimicas, 2002, 33, 3, 115-119.
UNK 841	9.82	-	0.18	-	-	m/z= 43, 74, 99, 57, 41, 72, 55, 45, 59, 73, 83, 80, 87, 56, 81, 54, 126 (M+)	841	-	-
UNK 848	10.00	0.02	0.08	0.09	0.08	m/z= 71, 43, 56, 59, 126 (M+ ?), 41, 111, 55, 67, 58, 93, 42, 53, 68, 61, 40, 72, 80	848	-	-
(Z)-2-hexenal	10.14	0.44	0.43	0.51	0.04	96	852	850	Mondello, L., HS-SPME-GCxGC-MS analysis of Yerba Mate (Ilex paraguariensis) in Shimadzu GC-GC application compendium of comprehensive 2D GC, Vol. 1-5, Shimadzu Corp., 2012, 1-29.
3-methyl-hexan-2-one	10.18	0.08	2.59	0.28	0.01	92	855	851	Sebastian, I.; Viallon-Fernandez, C.; Berge, P.; Berdague, J.-L., Analysis of the volatile fraction of lamb fat tissue: influence of the type of feeding, Sciences des Aliments, 2003, 23, 4, 497-511.
3-Penten-1-ol, 2-methyl-	10.28	-	1.19	-	0.04	90	859	-	-
UNK 862	10.38	-	0.19	-	0.05	m/z= 96, 81, 67, 53, 41, 95, 43, 55, 65, 40, 42, 51, 50, 70, 97, 68, 52, 66, 77, unknown M+	862	-	-
1-hexanol	10.49	15.63	1.43	-	-	97	866	865	Maia, J.G.S.; Andrade, E.H.A.; Zoghbi, M.G.B., Volatile constituents of the leaves, fruits and flowers of cashew (Anacardium occidentaleL.), J. Food Comp. Anal., 2000, 13, 3, 227-232.
UNK 881	10.87	-	0.07	-	-	m/z= 100, 85, 43, 82, 55, 83, 54, 114, 45, 53, 60, 99, 59, 41, 51, 50, 42, 101, 126 (M+)	881	-	-
UNK 899	11.35	-	9.01	-	-	m/z= 59, 88, 91, 60, 106(M+), 103, 61, 101, 94	899	-	-
UNK 902	11.44	-	3.97	-	-		902	-	-

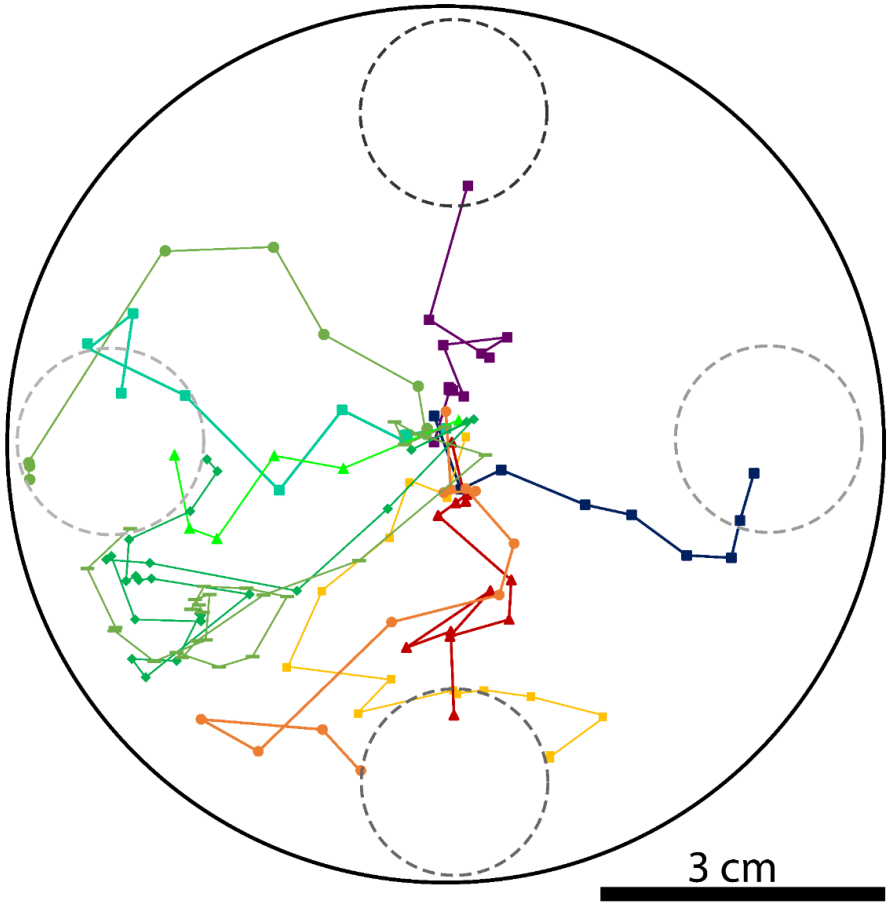
2,4 hexadienal	11.68	-	0.38	-	-	97	911	911	Campeol, E.; Flamini, G.; Cioni, P.L.; Morelli, I.; Cremonini, R.; Ceccarini, L., Volatile fractions from three cultivars of Olea eruopaea L. collected in two different seasons, J. Agric. Food Chem., 2003, 51, 7, 1994-1999.
pyrazine, 2,5-dimethyl-	11.76	-	0.00	0.27	-	94	912	911	Pino, J.A.; Mesa, J.; Muñoz, Y.; Martí, M.P.; Marbot, R., Volatile components from mango (Mangifera indica L.) cultivars, J. Agric. Food Chem., 2005, 53, 6, 2213-2223.
gamma-butyrolactone	11.87	-	0.00	2.25	-	86	915	915	Pino, J.A.; Mesa, J.; Muñoz, Y.; Martí, M.P.; Marbot, R., Volatile components from mango (Mangifera indica L.) cultivars, J. Agric. Food Chem., 2005, 53, 6, 2213-2223.
UNK 920 (59)	11.90	-	1.72	-	-	m/z= 43, 72, 84, 82, 83, 57, 44, 55, 41, 67, 71, 100(M+), 70, 42, 85	920	-	-
2,5 hexanedione	12.12	-	0.10	-	1.27	92	927	925	Poligné, I.; Collignan, A.; Trystram, G., Characterization of traditional processing of pork meat into boucané, Meat Sci., 2001, 59, 4, 377-389.
2-methylpentanoic acid	12.24	-	-	-	0.81	94	932	935	Radulovic, N.; Blagojevic, P.; Palic, R., Comparative study of the leaf volatiles of Arctostaphylos uva-ursi (L.) Spreng. and Vaccinium vitis-idaea L. (Ericaceae), Molecules, 2010, 15, 9, 6168-6185
UNK 937	12.38	-	1.13	-	-	m/z= 122 (M+ ?), 107, 121, 79, 77, 43, 53, 51, 81, 52	937	-	-
2-propanol, 1-butoxy-	12.51	-	-	-	3.14	96	942	947	Forero, M.D.; Quijano, C.E.; Pino, J.A., Volatile compounds of Chile pepper (Capsicum annuum L. var. glabriusculum) at two ripening stages, Flavour Fragr. J., 2008, 24, 1, 25-30.
UNK 955	12.88	-	2.18	-	-	m/z= 43, 69, 97, 58, 41, 85, 112, 59, 57, 54, 56, 70, 71, 53, 86, 95, 42, 55, 128(M+)	955	-	-
hept-2-enal	12.96	3.17	0.96	-	-	95	957	960	Weissbecker, B.; Holighaus, G.; Schütz, S., Gas chromatography with mass spectrometric and electroantennographic detection: analysis of wood odorants by direct coupling of insect olfaction and mass spectrometry, J. Chromatogr. A, 2004, 1056, 1-2, 209-216.
2 octanol	13.12	-	0.13	-	-	92	963	962	Nogueira, P.C.L.; Bittrich, V.; Shepherd, G.J.; Lopes, A.V.; Marsaioli, A.J., The ecological and taxonomic importance of flower volatiles of Clusia species (Guttiferae), Phytochemistry, 2001, 56, 5, 443-452.
1-heptanol	13.26	2.04	2.81	-	-	92	968	669	Pino, J.A.; Marbot, R.; Rosado, A.; Vázquez, C., Volatile constituents of Malay rose apple [Syzygium malaccense (L.) Merr. Perry], Flavour Fragr. J., 2004, 19, 1, 32-35.

hexanoic acid	13.31		0.39	-	4.93	94	972	977	Lalel, H.J.D.; Singh, Z.; Chye Tan, S., Glycosidically-bound aroma volatile compounds in the skin and pulp of 'Kensington Pride' mango fruit at different stages of maturity, Postharvest Biol. Technol., 2003, 29, 2, 205-218.
1-octen-3-ol	13.54	16.04	0.64	28.68	1.58	90	979	979	Chorianopoulos N.; Evergets E.; Mallouchos A.; Kalpoutzakis E.; Nychas G.J.; Haroutounian S.A., Characterization of the essential oil volatiles of Satureja thymbra and Satureja parnassica: Influence of harvesting time and antimicrobial activity, J. Agric. Food Chem., 2006, 54, 8, 3139-3145.
octane-2,3-dione	13.64	1.24	0.11	0.40	0.42	91	982	983	Methven L.; Tsoukka M.; Oruna-Concha M.J.; Parker J.K.; Mottram D.S., Influence of sulfur amino acids on the volatile and nonvolatile components of cooked salmon (Salmo salar), J. Agric. Food Chem., 2007, 55, 4, 1427-1436.
UNK 984	13.69	1.01	0.02	0.41		m/z= 68, 40, 93, 41, 110, 69, 94, 77, 55, 79, 57, 136 (M+)	985	-	-
6-methyl-5-hepten-2-one	13.77	3.82	21.13	15.81	9.11	96	987	987	Benzo, M.; Gilardoni, G.; Gandini, C.; Caccialanza, G.; Finzi, P.V.; Vidari, G.; Abdo, S.; Layedra, P., Determination of the threshold odor concentration of main odorants in essential oils using gas chromatography-olfactometry incremental dilution technique, J. Chromatogr. A, 2007, 1150, 1-2, 131-135
ethyl hexanoate	14.06	-	2.83	-	-	90	998	997	Nogueira, P.C.L.; Bittrich, V.; Shepherd, G.J.; Lopes, A.V.; Marsaioli, A.J., The ecological and taxonomic importance of flower volatiles of Clusia species (Guttiferae), Phytochemistry, 2001, 56, 5, 443-452.
2,4-heptadienal	14.43	1.12	1.32	-	-	94	1113	1113	Turchini, G.M.; Giani, I.; Caprino, F.; Moretti, V.M.; Valfrè, F., Discrimination of origin of farmed trout by means of biometrical parameters, fillet composition and flavor volatile compounds, Ital. J. Anim. Sci., 2004, 3, 123-140.
UNK 1020 (72, 142)	14.63	-	2.30	-	-	m/z= 72, 43, 71, 57, 142 (m+)	1020	-	-
2-ethyl-hexan-1-ol	14.87	-	1.61	-	10.31	97	1028	1029	Cho, I.H.; Namgung, H.-J.; Choi, H.-K.; Kim, Y.-S., Volatiles and key odorants in the pileus and stipe of pine-mushroom (Tricholoma matsutake Sing.), Food Chem., 2008, 106, 1, 71-76.
oct-3-en-2-one	15.21	3.85	-	-	-	95	1040	1040	Liu, S.; Lu, S.; Su, Y.; Guo, Y., Analysis of volatile compounds in Radix Bupleuri injection by GC-MS-MS, Chromatographia, 2011, 74, 5-6, 497-502.
2,3-dimethyl maleic anhydride	15.24	-	2.21	-	-	89	1043	1038	Brandi, F.; Bar, E.; Mourgues, F.; Horvath, G.; Turcsi, E.; Giuliano, G.; Liverani, A.; Tartarini, S.; Lewinsohn, E.; Rosati, C., Study of Redhaven peach and its white-fleshed

									mutant suggests a key role of CCD4 carotenoid dioxygenase in carotenoid and norisoprenoid volatile metabolism, BMC Plant Biol., 2011, 11, 24, 1-14
4-methyl-4-vinyl-1,4-butanolide	15.33	-	3.77	-	-	98	1046	1046	Avato, P.; Raffo, F.; Aldouri, N.A.; Vartanian, S.T., Essential oils of varthemia iphionoides from Jordan, Flavour Fragr. J., 2004, 19, 6, 559-561.
dihydro-2H-pyran-2-one	15.34	-	-	22.94	-	85	1043	1043	
terpene 1056	15.61	-	1.90	-	-	m/z= 69, 41, 95, 67, 82, 53, 55, 43, 70, 138 (m+), 109, 96	1056	-	-
2-octenal	15.72	-	1.40	-	-	96	1060	1060	Klesk, K.; Qian, M., Preliminary aroma comparison of Marion (Rubus spp. hyb) and Evergreen (R. laciniatus L.) blackberries by dynamic headspace/OSME technique, J. Food Sci., 2003, 68, 2, 697-700.
2,5-dimethyl-2,5-hexanediol	15.64	-	-	-	5.19	96	1060	1059	Klesk, K.; Qian, M., Preliminary aroma comparison of Marion (Rubus spp. hyb) and Evergreen (R. laciniatus L.) blackberries by dynamic headspace/OSME technique, J. Food Sci., 2003, 68, 2, 697-700.
methyl branced decane	15.76	2.79	-	9.90	-	95	1059	-	-
2-octen-1-ol	15.97	2.34	1.40	0.52	-	98	1068	1070	Jelen, H.H.; Grabarkiewicz-Szczesna, J., Volatile compounds of Aspergillus strains with different abilities to produce ochratoxin A, J. Agric. Food Chem., 2005, 53, 5, 1678-1683
octan-1-ol	16.00	3.15	2.23	2.41	-	92	1070	1069	Jelen, H.H.; Grabarkiewicz-Szczesna, J., Volatile compounds of Aspergillus strains with different abilities to produce ochratoxin A, J. Agric. Food Chem., 2005, 53, 5, 1678-1683.
3,5-octadien-2-one	16.05	-	1.23	-	-	91	1072	1072	Methven L.; Tsoukka M.; Oruna-Concha M.J.; Parker J.K.; Mottram D.S., Influence of sulfur amino acids on the volatile and nonvolatile components of cooked salmon (Salmo salar), J. Agric. Food Chem., 2007, 55, 4, 1427-1436.
cis-linalool oxide	16.21	-	2.06	-	12.43	94	1078	1078	Jalali-Heravi, M.; Zekavat, B.; Sereshti, H., Characterization of essential oil components of Iranian geranium oil using gas chromatography-mass spectrometry combined with chemometric resolution techniques, J. Chromatogr. A, 2006, 1114, 1, 154-163.
trans-linalool oxide	16.63	-	1.46	-	0.02	93	1094	1090	Blagojevic, P.; Radulovic, N.; Palic, R.; Stojanovic, G., Chemical composition of the essential oils of Serbian wild-growing Srtemisia absinthium and Artemisia vulgaris, J. Agric. Food Chem., 2006, 54, 13, 4780-4789.
UNK 1095	16.70	0.93	-	-	-	m/z= 95, 124 (M+), 79, 81, 85, 109, 43, 96, 41, 77, 65	1095	-	-

3,5-heptadien-2-one, 6-methyl-	17.00	-	2.19	-	12.44	95	1107	1107	Radulovic, N.; Blagojevic, P.; Palic, R., Comparative study of the leaf volatiles of Arctostaphylos uva-ursi (L.) Spreng. and Vaccinium vitis-idaea L. (Ericaceae), Molecules, 2010, 15, 9, 6168-6185.
UNK1109	17.07	-	1.74	-	8.95	m/z= 69, 41, 43, 112, 97, 71, 99, 98, 140 (M+), 83, 70, 125, 82, 57	1109	-	-
pyrazole derivate	17.16	-	1.05	-	4.11	m/z= 82, 81, 43, 54, 41, 59, 55, 83, 42, 59, 71, 97, 124 (M+ ?), 57	1113	-	-
UNK 1118	17.29	1.45	-	-	-	m/z= 71, 43, 95, 58, 41, 110, 57, 128, 55, 85, 40, 44, 69, 84, 70, 56, 72, 68, 42, 81, 67, 111, 113, 53, 96, 59, 156 (M+)	1118	-	-
UNK 1125	17.48	-	-	-	4.91	m/z= 57, 59, 43, 41, 72, 85, 71, 102, 44, 56, 105, 58, 55, 115, 156 (M+ ?)	1125	-	-
UNK 1151	18.16	1.81	-	-	-	m/z= 43, 87, 71, 70, 113, 95, 85, 156 (M+), 41, 58, 55, 57, 59, 86, 69, 110, 67, 98, 42, 100, 72, 44, 56, 122, 88	1151	-	-
1-nonanol	18.66	1.04	-	-	5.63	93	1171	1172	Wu, S.; Zorn, H.; Krings, U.; Berger, R.G., Volatiles from submerged and surface-cultured beefsteak fungus, Fistulina hepatica, Flavour Fragr. J., 2007, 22, 1, 53-60.
UNK ketone 1193	19.23	-	-	-	5.11	m/z= 43, 58, 84, 71, 101, 83, 57, 55, 59, 119, 85, 91, 41, 69, 72, 128, 134, 154, 166 (M+ ?)	1193	-	-

Movement patterns of *A. longisetosus*



7