The role of chemotactile cues in interspecific interactions among Central-European arthropod communities

Dissertation

zur Erlangung des Grades

Doktor der Naturwissenschaften

Am Fachbereich Biologie

der Johannes Gutenberg-Universität

in Mainz

Hellena Binz

geboren am 10.09.1984 in Bühl (Baden)

Mainz, 2014

Dekan: ██████████████

1. Berichterstatter: ████████████████
2. Berichterstatter: ███████████████

Datum der mündlichen Prüfung: 19.12.2014

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Summary

Organisms that inhabit the same ecosystem can influence each other either via direct interactions but also indirectly by emitting chemotactile cues in the environment. Behavioral changes induced by chemotactile cues can alter population dynamics and community structures equally strong as direct interactions. Their interest to ecologists has therefore increased rapidly in recent years. However, studies including multiple species to enlighten the overarching relevance of chemotactile cues are still rare. In my doctoral thesis I therefore investigated behavioral responses to chemotactile cues within interspecific predator-prey and competitor-competitor relationships among multiple and syntopic Mid-European arthropod species. By using different behavioral approaches, I provide empirical evidence that chemotactile cues can be used by several arthropods in favor of their own benefit. Moreover, my results show that behavioral changes are species-specific and depend on the respective life-history traits like e.g. body size, commonness or dominance rank of the species involved. I therefore assume that arthropods can detect and interpret chemotactile cues of their opponents but only implement behavioral responses if they trade-off otherwise incurred fitness costs. In summary, the results of my doctoral thesis show that it is of great importance to investigate the role of chemotactile cues within multi-species communities for being able to identify the underlying causes inducing behavioral responses. This in turn represents the basis to better understand the ecological relevance of chemotactile cues and their potential effects on ecosystem functions.

Zusammenfassung

In einem Ökosystem beeinflussen sich Tiere gegenseitig in erster Linie durch direkte Interaktionen. Ihr Verhalten kann aber auch indirekt durch chemotaktile Stoffe die andere Tiere in der Umwelt hinterlassen beeinflusst werden. Vergleichbar zu direkten Interaktionen können indirekt ausgelöste Verhaltensänderungen einen starken Einfluss auf Populationsdynamiken und Gemeinschaftsstrukturen eines Ökosystems haben. Obwohl das daran gehegte Interesse der Ökologen in den letzten Jahrzenten stark gestiegen ist, fehlen immer noch Studien, welche über mehrere Arten hinweg versuchen die übergreifende Relevanz von chemotaktilen Stoffen herauszufinden. Im Rahmen meiner Doktorarbeit untersuchte ich daher wie sich mehrere mitteleuropäische Arthropodenarten, abhängig von deren interspezifischen Räuber-Beute- und Konkurrenzbeziehungen, mittels chemotaktiler Stoffe beeinflussen können. Mithilfe unterschiedlicher Verhaltensversuche konnte ich empirisch nachweisen, dass verschiedene Arthropoden chemotaktile Stoffe zu ihrem eigenen Vorteil nutzen können. Außerdem zeigen meine Ergebnisse, dass die Verhaltensänderungen artspezifisch und abhängig von den jeweiligen Lebenszyklen und den damit verbundenen Eigenschaften (z.B. Körpergröße, Häufigkeit oder Rangordnung) der beteiligten Arten sind. Ich vermute daher, dass Arthropoden chemotaktile Stoffe ihrer Gegenspieler wahrnehmen und interpretieren können. Eine Verhaltensänderung scheint jedoch nur dann statt zu finden wenn ein Nichtreagieren starke Fitnesskosten mit sich führen würde. Zusammenfassend zeigen die Ergebnisse meiner Doktorarbeit, wie wichtig es ist, die Bedeutung von chemotaktilen Stoffen innerhalb vieler Arten einer Gemeinschaft zu testen, um die den Verhaltensänderungen zugrundeliegenden Ursachen identifizieren zu können. Dies wiederum stellt die Grundlage, um die ökologische Relevanz von chemotaktilen Stoffen und deren mögliche Effekte auf Ökosystemfunktionen besser zu verstehen.

General introduction

Interaction networks comprising multiple species, as well as their influence on ecosystem food web maintenance and community stability can be quite complex. It is thus important to determine distinct species interaction patterns and strategies for being able to understand the underlying mechanisms promoting their coexistence under limited resources. An important strategy which has been postulated to enhance species coexistence due to an increase of a species' likelihood to persist among competing populations is the adaptation of behavioral responses towards opponents (Kondoh 2007; Aránguiz-Acuña et al. 2011; Ikegawa et al. 2015). The resulting co-evolutionary arms race among the species concerned can in turn favor the evolutionary improvement of physiological abilities which allow the detection of different cues emitted by the opponents (Dawkins & Krebs 1979).

Direct cues like substrate vibrations (Hergenröder & Barth 1983; Evans et al. 2009), airborne vibrations (Tautz & Markl 1978; Gnatzy & Kämper 1990) and visual cues (Hunter et al. 2007) require a spatial proximity of the respective opponent. However, at that point, it might be already too late for the cue receiver to react in an efficient way. Organisms can thus also make use of chemotactile substances or metabolic products left by their opponent without any purpose such as hair or feathers (Ekner & Tryjanowski 2008), silk (Rypstra & Buddle 2013), urine (Baudoin et al. 2013), feces (Agarwala et al. 2003) or footprints (Eltz 2006), as well as intraspecific signals intentionally emitted by the opponent like scent marks (Hughes et al. 2010; Cárdenas et al. 2012) or sex pheromones (Wyatt 2003; Greenstone & Dickens 2005). These chemotactile cues do not necessarily require the presence of the cue emitter as they can persist for a certain time in the environment, indicating the recent residence of the opponent (Kats & Dill 1998). Moreover, chemotactile cues might transmit specific information about an individual's life history (e.g. taxonomic affiliation (Jędrzejewski et al. 1993), size (Persons & Rypstra, 2001) or individual activity (Miller et al. 2013)) which is prerequisite for the deployment of species-specific defenses.

The adaptation of behavioral responses towards chemotactile cues from opponents can therefore be advantageous in at least two different ways: (1) the responding organism can increase its personal fitness by a faster acquisition of information (Ferrari et al. 2010; Lichtenberg et al. 2011) and thus maintain coexistence due to an increased propensity of local persistence; (2) Species diversity and thus number of coexisting species in an ecosystem is limited by the available resources. By possessing a combination of different physiological and behavioral traits, species differentiate into species-specific niches (Chase & Leibold 2003). The ability to respond to chemotactile cues may thus be an additional behavioral trait influencing niche differentiation between the species involved.

Antipredator behavior of prey species

In the last decade, studies focusing on behavioral changes caused by chemotactile cues have mainly focused on potential prey species because the performed adaptive behavioral response (i.e. antipredator behavior) can strongly influence the outcome of trophic interactions (Martinou et al. 2009; Schmidt-Entling & Siegenthaler 2009). By cascading down to lower trophic levels, like for example herbivores, it can have strong effects on ecosystem functions (Schmitz et al. 1997). Such non-consumptive effects can thus alter plant productivity, energy flux and nutrient cycling (Schmitz et al. 2008; Hawlena et al. 2012), as well as community dynamics (Werner & Peacor 2003; Abrams 2007). However, species-specific antipredator behavior can also strongly promote community stability (Kondoh 2007), by neglecting behavioral responses towards less abundant predators. As prey species have to balance the risk of predation to the incurred fitness costs (e.g. reduced feeding time or reproduction, Lima & Bednekoff 1999; Lind & Cresswell 2005), the strength, direction and number of the induced antipredator behaviors should be adjusted to the degree and type of threat a predator poses to the prey (Helfman 1989). Prey species may thus generate a minority-advantage mechanism and enhance the coexistence of the competing predators (Matsuda et al. 1993). Species-specific traits of both predator and prey, like for example body size (Persons & Rypstra 2001), hunting mode (Miller et al. 2013) or degree of

specialization (Jędrzejewski et al. 1993) may thereby additionally affect the outcome of a preys' antipredator behavior and ultimately interspecific population dynamics.

Given the importance and potential of chemotactile cue induced antipredator behaviors it is not surprising that their interest to ecologists has increased rapidly during the last years (Preisser & Bolnick 2008a; Preisser et al. 2009; Jones & Dornhaus 2011; Johnson & Sullivan 2014). However, existing studies mostly included only few prey species and a limited number of predator species (e.g. Jędrzejewski et al. 1993; Storm & Lima 2008, 2010; Miller et al. 2013). The overall effect of predator chemotactile cues on different potential prey species and their potential role in maintaining species coexistence is therefore still unclear. To enlighten their impact on species community structures, multispecies approaches, involving taxonomically different but syntopic species are thus needed.

Chemotactile cue induced prey search in predators

For an optimal foraging success, predators generally forage in a frequency-dependent way. As a consequence, they disproportionately favor frequently encountered prey over rare, which regulates prey species coexisting by exerting apparent competition (Ishii & Shimada 2012; Křivan 2013). As abundance can also be linked to dominance, predators may prevent dominant prey species from gaining prevalence (Walls et al. 2002). However, as a consequence to the predator's adaptive diet choice, frequently encountered prey may evolve higher antipredator defenses which would in turn reduce the predators' foraging success (Yamauchi & Yamamura 2005). Predators can thus drastically increase their foraging efficiency, by responding to chemotactile prey cues (Hughes et al. 2010; Telfer & Laberge 2013), also when searching for camouflaged or randomly scattered prey (Schatz et al. 2003; Carthey et al. 2011). Various mammals like for example weasels use their prey's intraspecific scent marks or bedding odors to facilitate prey localization (Sundell et al. 2003). Herbivore-induced volatiles, emitted by the preys host plant, are also used for prey search in a variety of hymenopteran parasitoids (Clavijo McCormick et al. 2012). However, these predators are mainly specialized on a narrow range of prey species as they have to learn or adapt to preys'

chemotactile cues. The limited reception capability for multiple cues was also shown in the ant-eating spider *Zodarion rubidium*. Although it consumes at least five different ant species, airborne cues of only two ant species provoked an attraction in the spider (Cárdenas et al. 2012). A similar pattern could also be observed in the recruitment behavior of the obligate termite-eating ant *Megaponera foetens*. Here, the ant only recruited their nestmates to chemical cues of two out of four potential prey species (Longhurst & Howse 1978). For generalist predators it could therefore be difficult to learn or adapt to chemotactile cues of all their potential prey species.

Nevertheless, due to their indiscriminate prey search, generalist predators represent important ecosystem agents: By reducing overall prey abundance they also reduce the intensity of prey interspecific competition which not only maintain prey species coexistence but can even increase species diversity among sites (Ryberg et al. 2012). Yet, to our knowledge, up to now empirical studies testing the response of generalist predators to chemotactile cues of taxonomically distinct prey species are still lacking. Therefore it remains unclear whether generalist predators use olfactory cues of potential prey species in prey search in general, whether their responses differ between distinct types of prey and/or ultimately its implication for species diversity.

Chemotactile cues shaping interspecific competition

Co-existing species that compete for the same resources are common and widespread and, like predator-prey relationships, also strongly influence population dynamics and community structure by exerting a high selection pressure on the species involved (Gibb & Johansson 2011; Dhondt 2012). In particular, dominant species aim to maintain their supremacy by aggressively displacing inferior species from established territories (Rowland 1983) and from shared food sources, even if initially encountered by subordinates (Drescher et al. 2011). To counteract their potential fitness costs, inferior species can therefore use the advantage of chemotactile cues emitted by dominants. By avoiding areas of high risk they can circumvent energy costly direct aggressive interference with dominant competitors (Baudoin et al. 2013). However, also dominant species can raise their fitness by exploiting chemotactile cues of inferior species. Like chemically orienting predators use chemotactile cues in prey search, dominant

competitors may save their own time searching by following chemotactile cues of subordinates to either prey upon them (Agarwala et al. 2003) or in order to steal their food (kleptoparasitism: Creel et al. 2001; Hunter et al. 2007). The two competing stingless bees *Melipona rufiventris* and *Trigona spinipes* are a good example of a chemotactile shaped interspecific competition: The dominant *T. spinipes* seeks flowers with chemotactile cues of the inferior *M. rufiventris* to forcefully take over the food source. Hence, the latter avoids flowers previously visited by its dominant competitor *T. spinipes* (Nieh et al. 2004). The exploitation of chemotactile cues among competing animals could represent an additional functional trait which promotes niche differentiation among the competing species and thus an additional mechanism enhancing species coexistence and local species richness (Chesson 2000). Nonetheless, studies on the role of chemotactile cues in interspecific interactions are still rare and involved only two competitors at a time (Agarwala et al. 2003; Polo-Cavia et al. 2009; Lichtenberg et al. 2011).

Chemotactile cues in arthropod communities

Arthropods and especially the order Insecta therein, are the most numerous phylum in the world (Hill 1997). From small zooplankton up to predators: arthropods occupy almost all imaginable niches in the world and accomplish a great number of important ecosystem services (Prather et al. 2013). In both, aquatic and terrestrial ecosystems, arthropods represent the vast majority of animals that communicate chemically (Greenfield 2002) and the implementation of chemotactile cues in arthropod interspecific interactions seems thus natural. Indeed, former studies could already show that chemotactile cues can be used by arthropods to shape the outcome of interactions (e.g. daphnia (Conde-Porcuna 1998; Roozen & Lüderling 2001), spiders (Clark et al. 2000; Persons & Rypstra 2001; Schonewolf et al. 2006), crickets (Kortet & Hedrick 2004; Storm & Lima 2008), stingless bees (Nieh et al. 2004), bumblebees (Dawson & Chittka 2012), ants (Menzel et al. 2010a) and beetles (Godeau et al. 2003; Agarwala et al. 2003)). However, there is a strong bias towards aquatic ecosystems (Van Donk 2007; Preisser & Bolnick 2008b; Hay 2009; Ferrari et al. 2010) and studies

that focused on terrestrial ecosystems were limited to only few species at a time. Thus, the overarching role of chemotactile cues for terrestrial arthropod communities remains open.

Research Goals

My dissertation research aims to fill current research gaps on the importance of chemotactile cues in shaping multispecies interactions. By studying behavioral responses towards chemotactile cues in predator-prey and competitor interactions among Central-European arthropod communities, we addressed the following aspects: (1) the commonness of prey behavioral changes upon the detection of predator chemotactile cues, (2) identification of predator life history traits that shape prey behavioral changes, (3) the role of prey chemotactile cues for generalist predators during prey search and (4) the influence of dominance rank on chemotactile cue exploitation in interspecific competition.

Publication 1

How widespread are antipredator behaviors of potential arthropod prey species towards chemotactile cues of their generalist predators?

Spiders are diverse and abundant generalist predators, preying upon insects but also other spiders, hence they play an important role in shaping terrestrial ecosystems (Wise 1993; Nentwig 2013). Nonetheless, chemotactile cue induced antipredator behavior has only been studied in few potential prey taxa so far. We therefore studied antipredator behaviors of 13 insect and eight spider species from a wide taxonomic range in response to chemotactile cues of three common spider species. Prey species were confronted with predator chemotactile cues in two different arena experiments: In the first experiment prey individuals were either put on filter papers bearing spider cues or on cue-free filter papers in two separate arenas. In the second experiment, prey individuals were allowed to choose between the two filter paper halves in the same arena. Prey activity patterns were afterwards analyzed using automated video analysis.

Publication 2

Are the strength and direction of antipredator behavior influenced by different predator life-history traits?

Spiders possess a variety of life-history traits that may shape the outcome of prey antipredator behaviors (e.g. Persons & Rypstra 2001; Miller et al. 2013). Using similar methods as described above we tested antipredator responses of the wood cricket *Nemobius sylvestris* when confronted with chemotactile cues of fourteen syntopic spider species that strongly varied in their life-history traits. We could thus test for an effect of predator hunting mode, predator commonness, predator diurnal activity and predator-prey body size ratio on wood cricket antipredator behaviors. Additionally, we tested if strength and direction of preys' antipredator behavior depends on the current developmental life stages of predator and prey.

Publication 3

Are generalist predators able to detect and respond to chemotactile cues of taxonomically distinct prey species?

Ants are ubiquitous and common generalist predators on arthropods and affect the functioning of most terrestrial ecosystems (Folgarait 1998; Domisch et al. 2009). They possess highly sophisticated olfactory abilities and communicate prevalent chemically. We investigated behavioral responses of the wood ant *Formica polyctena* towards chemotactile cues of two taxonomically distinct but potential prey species and, in analogy, the responses of the two prey species to ant cues. The respective responses were tested in arena experiments where individuals were able to choose between cue bearing and cue free filter paper halves. Additional cafeteria tests were conducted in the field, testing how predator responses to prey cues coincide with prey preferences. Furthermore, aggression tests were performed to test if prey responses to predator cues correspond to predator threat.

Publication 4

Can competing arthropod species exploit chemotactile cues of their opponents and how does it depend on their rank in the local dominance hierarchy?

Among arthropods, interspecific competition is particularly pronounced in ant communities (Parr & Gibb 2012) which are therefore usually structured into dominance hierarchies (Fellers 1987; Parr & Gibb 2010). To investigate the role of chemotactile cues, we first examined the respective dominance hierarchy among five co-existing ant species using aggression assays. Behavioral responses to chemotactile cues were then tested using Y-maze assays with one arm bearing cues and one clean arm. First we tested responses towards cuticular hydrocarbons extracts between dominant and subordinate species. Afterwards, we tested the responses of subordinate species to trail pheromone extracts of dominants. Finally, we studied whether the lower quantities of dominants' footprints, in contrast to cuticular hydrocarbon extracts, induce a less pronounced response in subordinates.

1.

Effects of spider cues on arthropod behavior

Nursery web spider (*Pisaura mirabilis*) on a filter paper, donating cues for further arena experiments.

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This chapter is based on an original research article published in the *Journal of Insect Behavior* in April 2014:

Bucher R., Binz H., Menzel F. and Entling M.H. 2014. Effects of spider Chemotactile Cues on Arthropod Behavior. *J. Insect Behav.* **27, 567-580.**

1.1. Abstract

Predation risk can strongly affect the behavior of prey species. However, empirical evidence for changes in behavior driven by spider cues is restricted to relatively few taxa. Here, we conducted a series of behavioral experiments to test for changes in activity among a wide range of terrestrial arthropods. We confronted 13 insect and eight spider species with chemotactile cues of three spider species. We applied two different experimental setups: In the 'no-choice experiment' prey individuals were either put on control filter papers or on filter papers previously occupied by a spider. In the 'choice experiment', the prey individuals were able to choose between filter paper halves with and without spider cues. In both setups, the response to spider cues depended significantly on prey species, with some species increasing and others decreasing their activity. Surprisingly few prey species responded to the spider cues at all. Our results indicate that predator recognition upon contact with cue bearing filter papers is strongly prey-specific and that behavioral effects driven by spider chemotactile cues are an exception rather than the rule among terrestrial arthropods.

1.2. Introduction

Besides consumption of prey, predators can modify prey traits such as morphology, reproductive strategy, or behavior. A variety of terms have been used to subsume these effects (Werner & Peacor 2003; Abrams 2007) To describe direct effects of predators on species traits we favor the term non-consumptive effects, following the suggestions by P. A. Abrams (2007). Originally, the term non-consumptive effects derived from predator-prey interactions. However, intraguild interactions share the characteristics of non-consumptive effects as long as they concern direct interference, but exclude consumption among intraguild predators.

Here, we focus on behavioral effects upon contact with predator chemotactile cues. Several organisms recognize predator cues and change their behavior accordingly (Kats & Dill 1998). In doing so, prey individuals can show two basic strategies to avoid predation: Either they speed up to directly escape predation and reach predator-free space or they reduce their mobility ("freezing") to lower the detection probability by predators (Lima & Dill 1990). Both strategies are expected to enhance individual survival but they bear additional costs (Lima & Dill 1990; Preisser & Bolnick 2008b). The strength and direction of behavioral changes can depend on encounter probability (Binz et al. 2014b), predator-prey size ratio (Preisser & Orrock 2012), prey sex (Sitvarin & Rypstra 2012), hunger level (Walker & Rypstra 2003), and predator hunting mode (Miller et al. 2013). Relatively larger predators for example are expected to induce stronger behavioral changes compared to smaller ones (Persons & Rypstra 2001), and cues of more sedentary predators (i.e. sit-and-wait, sit-and-pursue) evoke stronger non-consumptive effects than actively hunting predators (Preisser et al. 2007). Behavioral effects driven by predator cues can interfere with other behaviors like mate choice (Hedrick & Dill 1993) or foraging (Schmitz & Suttle 2001). Changes in foraging behavior are especially crucial, because they cascade down to lower trophic levels such as herbivores (Martinou et al. 2009; Schmidt-Entling & Siegenthaler 2009) or plants (Messina 1981; Schmitz et al. 2000; Steffan & Snyder 2010). Consequently, non-consumptive effects, and behavioral effects in particular, can play an important role in ecosystem functioning (Schmitz et al. 1997).

In terms of taxonomic groups, non-consumptive effects have been studied in a wide range of organisms (Lima & Dill 1990; Kats & Dill 1998; Caro 2005). However, there is still a bias towards aquatic systems, while terrestrial systems are underrepresented in the literature (Kats & Dill 1998; Preisser et al. 2005; Preisser & Bolnick 2008b). Regarding terrestrial invertebrates, researchers have often used spiders to demonstrate evidence for non-consumptive effects of predators (e.g. Schmitz 1998; Persons et al. 2001; Storm & Lima 2008; Schmidt-Entling & Siegenthaler 2009; Rypstra & Buddle 2013). However, current studies of non-consumptive effects elicited by spiders include mainly orthopteran prey (Beckerman et al. 1997; Schmitz & Suttle 2001; Danner & Joern 2003; Kortet & Hedrick 2004; Storm & Lima 2008). Effects of spiders on collembolans and beetles have also been reported (Snyder & Wise 2000; Grear & Schmitz 2005; Rypstra & Buddle 2013). Another important part of the literature focuses on non-consumptive effects of spiders on other predators (Persons et al. 2001; Li 2002; Schonewolf et al. 2006; Whitehouse et al. 2011). Such effects may be of wide relevance because intraguild interference is particularly common in terrestrial ecosystems (Vance-Chalcraft et al. 2007), and because community-wide studies found that non-consumptive effects on intermediate predators can be particularly strong (Moran & Hurd 1994; Schmidt-Entling & Siegenthaler 2009). Different prey species use different antipredator strategies even against the same predator (Caro 2005). Still, existing studies comprise only a small number of prey species and multispecies approaches are scarce. Due to the publication of single species combinations, non-significant results in further species combinations may remain unpublished. To account for the wide prey spectrum of generalist predators, large-scale comparisons across broad ranges of prey taxa are crucial to reveal the general occurrence and importance of non-consumptive effects among groups that are underrepresented yet.

Our aim was to study the occurrence and strength of behavioral responses towards predator cues among terrestrial arthropods across a wide taxonomic range. We directly compared behavior of 21 different insect and spider species in response to chemotactile cues of three spider species that are potential predators or competitors of the above species. To determine effects of predator and prey identity on the magnitude

of behavioral changes in response to cue-bearing filter paper we performed two parallel experimental series. In the 'no-choice experiment', prey individuals were either put on cue-bearing filter papers or on cue-free filter papers serving as control. In the 'choice experiment', prey individuals were allowed to choose between cue-bearing and cue-free filter paper halves. The two experimental setups have been applied in former studies (Persons et al. 2001; Storm & Lima 2008) to test for behavioral changes in response to predator cues. Significant behavioral changes would indicate that individuals recognized predators via their chemotactile cues and responded accordingly. The behavioral response could be interpreted as predator avoidance (i.e. antipredator behavior) or avoidance of competitors depending on the specific relationship. Based on the strength of behavioral effects previously reported, we expected clear behavioral changes among the tested species combinations.

1.3. Methods

Study species

Arthropods were captured in the field between June and September 2011 at two sites around the city of Landau (Rhineland-Palatinate, Germany): heath-like open vegetation adjacent to a mixed forest near Bellheim (49°11'45" N / 8°19'11" E), and a heterogeneous fallow near Gleishorbach (49°07'25" N / 7°59'34" E). The predator species were chosen based on preliminary results of an earlier study about non-consumptive effect of various spider species on wood crickets (Binz et al. 2014b). We used four spider species that were common at both sites: *Aulonia albimana* (WALCKENAER, 1805; Lycosidae), *Agelena labyrinthica* (CLERCK, 1757; Agelenidae) or *Allagelena gracilens* (C. L. KOCH, 1841; Agelenidae), and *Pisaura mirabilis* (CLERCK, 1757; Pisauridae) (Table 1.1). During our experiments we had to switch between the species *Agelena labyrinthica* and *Allagelena gracilens*, because *Agelena labyrinthica* became unavailable in the field in late summer. Both species are similar in many respects (e.g. body size and hunting mode) with the exception of their seasonal occurrence. We excluded adult males, because in most spider species males do no longer hunt for prey once they reach adulthood (Foelix 2011). Both *Aulonia albimana* and both species of the family Agelenidae build funnel webs in the vegetation close to the ground (Roberts

1996). *Aulonia albimana* is also actively hunting outside the web (Job 1974). In contrast, *Pisaura mirabilis* can be categorized as sit-and-wait predator that prefers hunting on vegetation (Roberts 1996).

Table 1.1: Predator species, their hunting mode, life-stage (immature/adult), sex (juvenile/female), and body length (mm) of N individuals measured for the experiments. Note that because *Agelena labyrinthica* became unavailable in the field, we had to switch to *Allagelena gracilens* during our experiments.

Family	Species	Hunting mode	Stage	Sex	Length	N
Pisauridae	*Pisaura mirabilis*	sit-and-wait	im-ad	j-ff	5.7 ±0.9	315
Agelenidae	*Agelena labyrinthica*	web	ad	ff	8.5 ±0.5	165
	Allagelena gracilens	web	ad	ff	8.9 ±0.9	150
Lycosidae	*Aulonia albimana*	web/active	ad	ff	4.3 ±0.5	315

For prey (including intraguild prey) we used 13 insect and eight spider species that were very common at either of our two sites at the same time period we collected our predator species (with exception of the fruit fly *Drosophila hydei*, which were cultured in the lab). The set of prey species represented various taxonomic and functional groups (Table 1.2). We collected live spiders and insects with pitfall traps, suction sampling, and by hand. Predators were maintained singly in 40 ml glasses with moistened plaster of Paris at 16 °C, 65% humidity, and 12/12 h day/night rhythm in a climatic chamber. The predators were fed with fruit flies (*Drosophila hydei*) twice a week (with three to five individuals depending on the size). We consider the nutritional status of the predators as an intermediate hunger level. Spiders and carnivorous insects that we used as prey were fed in the same rhythm with either springtails (*Sinella curviseta*) or fruit flies (*Drosophila hydei*). Omnivorous insects were fed with apple and carrots *ad libitum*. Herbivores were maintained in groups on their host plants in plastic terraria (30 x 20 x 20 cm). Prey organisms were kept in the climate chamber for at least one week prior to the experiments for acclimatization (e.g. to reduce individual variation). After that, the experimental series were performed within few days to reduce temporal variation.

Cue preparation

Predator cues were obtained by keeping spiders on moistened filter papers for 24 hours (one spider per filter paper) in a climate cabinet (25 °C and 75 % humidity) prior to the experiment. Chemotactile cues consisted of feces droplets, chemical footprints (traces of chemical substances associated with spider tarsi) and/or dragline silk. The moistening prior to cue deposition prevented spiders from drying out and enhanced the adherence of filter papers to the petri dishes, which prevented prey individuals from hiding under the filter papers. The spiders were removed directly before the behavioral experiments. The restricted volume of the petri dishes prevents especially spiders of the family Agelenidae from building extensive/functional webs. Three dimensional webs structures were removed if present while transferring filter papers to the experimental arenas, because these structures would have physically reduced mobility on cue-bearing filter papers.

No-choice experiment

To test for effects of prey identity on the magnitude of behavioral changes, we confronted each of 21 prey species (Table 1.2) with chemotactile cues from each of the three predator species (*Aulonia albimana*, *Agelena labyrinthica* or *Allagelena gracilens*, and *Pisaura mirabilis*). For the no-choice experiment, we covered the bottom of experimental arenas (petri dish covered with a plastic tube 10 cm diameter and 15 cm height coated with fluon) with filter papers (9 cm diameter). A new petri dish and filter paper was used for each experiment. For each of the combinations we used ten filter papers with chemotactile cues. Another ten filter papers without predator cues served as control resulting in 3 predator species × 21 prey species × 20 arenas (i.e.: 10 replicates per treatment) = 1260 trials. The arenas of each combination were randomly compiled and five of them were filmed at the same time with a video camera (SONY HDR-CX 550 VE) mounted on a tripod. The prey individual was released into the center of the arena, and filmed for 15 minutes. During the video takes, we covered the arenas with glass panels to exclude intermixing of possible volatile cues among the arenas. All experiments were conducted in the lab at 23.5 °C and 30 % humidity (see supplementary material S1 for drawings of the experimental setups).

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Table1.2: Prey species, their trophic position, life-stage (immature/adult), sex (not specified/juvenile/unfertile female/ fertile female), and body length (mm) of 90 individuals per species at the time of the experiment. Movement (Mov.) indicates the time spent moving relative to the total duration of the recordings across all predator combinations and experimental setups.

Group	Species	Trophic pos.	Stage	Sex	Length	Mov.
Collembola	*Tomocerus longicornis*	detritivore	im-ad	-	4.3 ±0.7	12 %
Dermaptera	*Forficula auricularia*	omnivore	im-ad	-	13.9 ±2.0	33 %
Blattodea	*Ectobius sylvestris*	omnivore	im-ad	-	6.6 ±1.9	26 %
Orthoptera	*Nemobius sylvestris*	omnivore	im	-	5.4 ±1.2	30 %
	Chorthippus dorsatus	herbivore	im-ad	-	18.0 ±3.4	19 %
Heteroptera	*Nabis rugosus*	carnivore	im-ad	-	6.1 ±0.4	15 %
Coleoptera	*Paederus littoralis*	carnivore	ad	-	8.3 ±0.7	59 %
	Sitona lineatus	herbivore	ad	-	5.1 ±0.4	31 %
	Psyllobora 22-*punctata*	fungivore	ad	-	3.4 ±0.3	52 %
	Phyllotreta sp.	herbivore	ad	-	2.1 ±0.2	20 %
Hymenoptera	*Tenthredopsis* sp.	herbivore	im	-	10.1 ±2.0	32 %
	Lasius niger	omnivore	ad	f	3.1 ±0.3	42 %
Diptera	*Drosophila hydei*	herbivore	ad	-	3.0 ±0.2	32 %
Thomisidae	*Xysticus cristatus*	carnivore	im-ad	j-ff	4.1 ±1.1	32 %
Agelenidae	*Allagelena gracilens*	carnivore	ad	ff	8.8 ±0.9	39 %
Lycosidae	*Aulonia albimana*	carnivore	ad	ff	4.1 ±0.4	32 %
	Pardosa lugubris-group	carnivore	ad	ff	5.2 ±0.7	17 %
	Alopecosa pulverulenta	carnivore	im-ad	j-ff	5.2 ±0.6	30 %
Pisauridae	*Pisaura mirabilis*	carnivore	im-ad	j-ff	5.9 ±0.7	17 %
Linyphiidae	*Erigone dentipalpis*	carnivore	ad	ff	2.1 ±0.2	25 %
Tetragnathidae	*Pachygnatha degeeri*	carnivore	im-ad	j-ff	3.5 ±0.4	42 %

Choice experiment

In addition to the no-choice experiment, where prey species were put either in arenas with spider cues or without cues (control), we conducted series of choice experiments (with the same predator-prey combinations). In the choice setup prey individuals were able to choose between control and cue-bearing filter paper halves. Here, we covered ten experimental arenas with two semi-circles of filter paper, one previously occupied by a spider and one without spider cues. In this second experiment, we performed 3 predator species × 21 prey species × 10 arenas = 630 trials. The filming procedure was

similar to the previous experiment. In the choice experiment, possible volatile cues could diffuse into the initially cue-free zone of the arena. This may mitigate differences between the treatments. Only in the control arena of the no-choice setup we can properly exclude the presence of any predator cues. This is especially important, because the chemical compounds of spider cues that mediate such behavioral effects have not been identified yet (Schulz 2004).

Video analysis and statistics

We analyzed our videos using the software EthoVision XT 8 (Noldus Information Technology, Wageningen, The Netherlands) with the Multiple Arenas Module to track the five prey individuals simultaneously. The following behavioral parameters were measured: Distance travelled, time spent immobile vs. time spent mobile (threshold between 5% and 60% pixel changes per frame of the area detected as prey) vs. time spent highly mobile (more than 60% pixel changes); time spent moving (central point of the individual is moving in a directed manner) vs. time spent not moving. In addition, for the choice experiment, we recorded the duration of stay on the control and the cue half of the filter paper. Most of the parameters were highly correlated. Therefore, we reduced the movement parameters using principal component analysis (see supplementary material S2 for more details about the reduction of the movement variables). The scores of the first principal component were used as a measure of activity.

Statistical tests were done in two steps. First, we used a linear mixed model to test for overall effects of the cue treatment and the interaction of cue treatment with predator and prey identity. For the no-choice experiment, predator-prey combination entered the model as random factor. In a second step, we tested for changes in prey behavior for each predator-prey combination. For this, we compared prey activity between cue-bearing and cue-less (control) filter paper using two-sided t-tests. We did not adjust the p-values of the single predator-prey combinations for multiple testing in order to avoid the inflation of type II-errors (García 2004). For the choice experiment we corrected movement parameters for residence time on the respective filter paper half (i.e. proportional data) prior to variable reduction. Comparable to the above

approach, data from the choice experiment were first tested for overall significance and then in each predator-prey combination. For the overall test, we tested activity against the treatment and the interaction of the treatment with predator and prey identity using a linear mixed model nested for individual prey. For the predator-prey combinations we calculated the differences in the movement parameters between the two treatments in the same arena. We then tested differences in activity between cue and control filter paper against zero using one-sample t-tests. All statistical analyses were calculated in R version (2.14.1) (R Development Core Team 2011).

1.4. Results

No-choice experiment

Contrary to our expectation, behavioral changes in response to spider chemotactile cues were not overall significant ($F_{1,1124}$ = 0.05, p = 0.83). Nonetheless, we found a highly significant interaction between treatment and prey species ($F_{40,1124}$ = 7.09, $p < 0.0001$) indicating that behavioral changes upon contact with spider chemotactile cues are prey specific. The interaction between treatment and predator was not significant ($F_{4,1124}$ = 1.19, p = 0.31). Out of the 21 tested prey species, only one spider and two insects responded significantly to predator chemotactile cues (Fig. 1.1). The wood cricket *Nemobius sylvestris* (BOSC, 1792; Gryllidae) was significantly more active in the presence of cues of *Pisaura mirabilis* (t_{18} = 2.43, p = 0.03) and *Allagelena gracilens* (t_{17} = 2.57, p = 0.02) but not when confronted with cues of the smaller wolf spider *Aulonia albimana* (t_{15} = -1.58, p = 0.14). The ant *Lasius niger* (LINNAEUS, 1758; Formicidae) was significantly more active in the presence of cues of *Aulonia albimana* (t_{18} = 2.34, p = 0.03) but did not react to cues of the other two spiders (*Pisaura mirabilis*: t_{18} = 0.55, p = 0.59; *Allagelena gracilens*: t_{17} = 0.49, p = 0.63). The spider *Pisaura mirabilis* became less active on filter papers previously occupied by conspecifics (t_{15} = -2.77, p = 0.014). Among the remaining predator prey combinations, we detected no significant response to spider chemotactile cues.

Choice experiment

Similar to the no-choice experiment changes in activity upon contact with chemotactile cues of the three spider species were not overall significant ($F_{1,576}$ = 0.49, p = 0.48). But

again the interaction between treatment and prey identity was highly significant ($F_{40,576}$ = 15.52, p < 0.0001). The interaction between predator and treatment was again not significant ($F_{4,576}$ = 1.54, p = 0.19). Among the single predator-prey combinations, three insect species showed significant responses to predator chemotactile cues (Fig. 1.2). In the choice experiment wood crickets *Nemobius sylvestris* were more active in the presence of cues of *Aulonia albimana* (t_9 = 2.51, p = 0.03) but not in the presence of cues of the remaining two predators (*Pisaura mirabilis*: t_9 = -1.49, p = 0.17; *Allagelena gracilens*: t_9 = -1.39, p = 0.20). Similar to the no-choice experiment, the ant *Lasius niger* showed higher activity in the presence of cues from *Aulonia albimana* (t_9 = 2.64, p = 0.03), but again not on cues of the two other spider species (*Pisaura mirabilis*: t_9 = -0.89, p = 0.40; *Allagelena gracilens*: t_9 = 0.85, p = 0.42). In addition, the cockroach *Ectobius sylvestris* (BRUNNER VON WATTENWYL, 1865; Ectobiidae) was less active on cues of *Pisaura mirabilis* (t_9 = -2.49, p = 0.04).
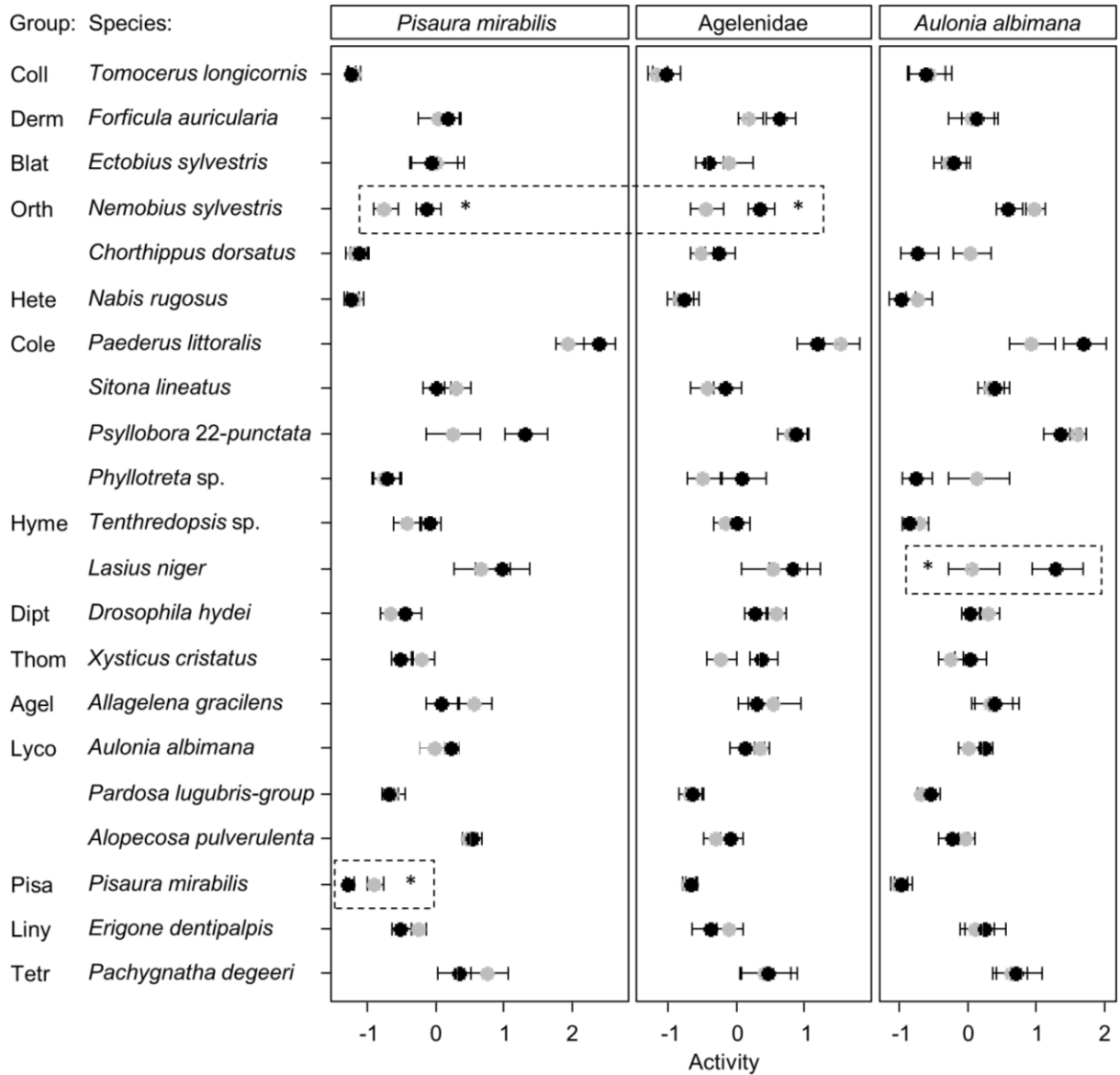
Figure 1.1: Prey activity (mean ± se) on control filter papers (grey dots) and filter papers bearing chemotactile cues (black dots) of the three predators in the no choice setup ($p < 0.05$ *). (Coll = Collembola, Derm = Dermaptera, Blat = Blattodea, Orth = Orthoptera, Hete = Heteroptera, Cole = Coleoptera, Hyme = Hymenoptera, Dipt = Diptera, Thom = Thomisidae, Agel = Agelenidae, Lyco = Lycosidae, Pisa = Pisauridae, Liny = Linyphiidae, and Tetr = Tetragnathidae).
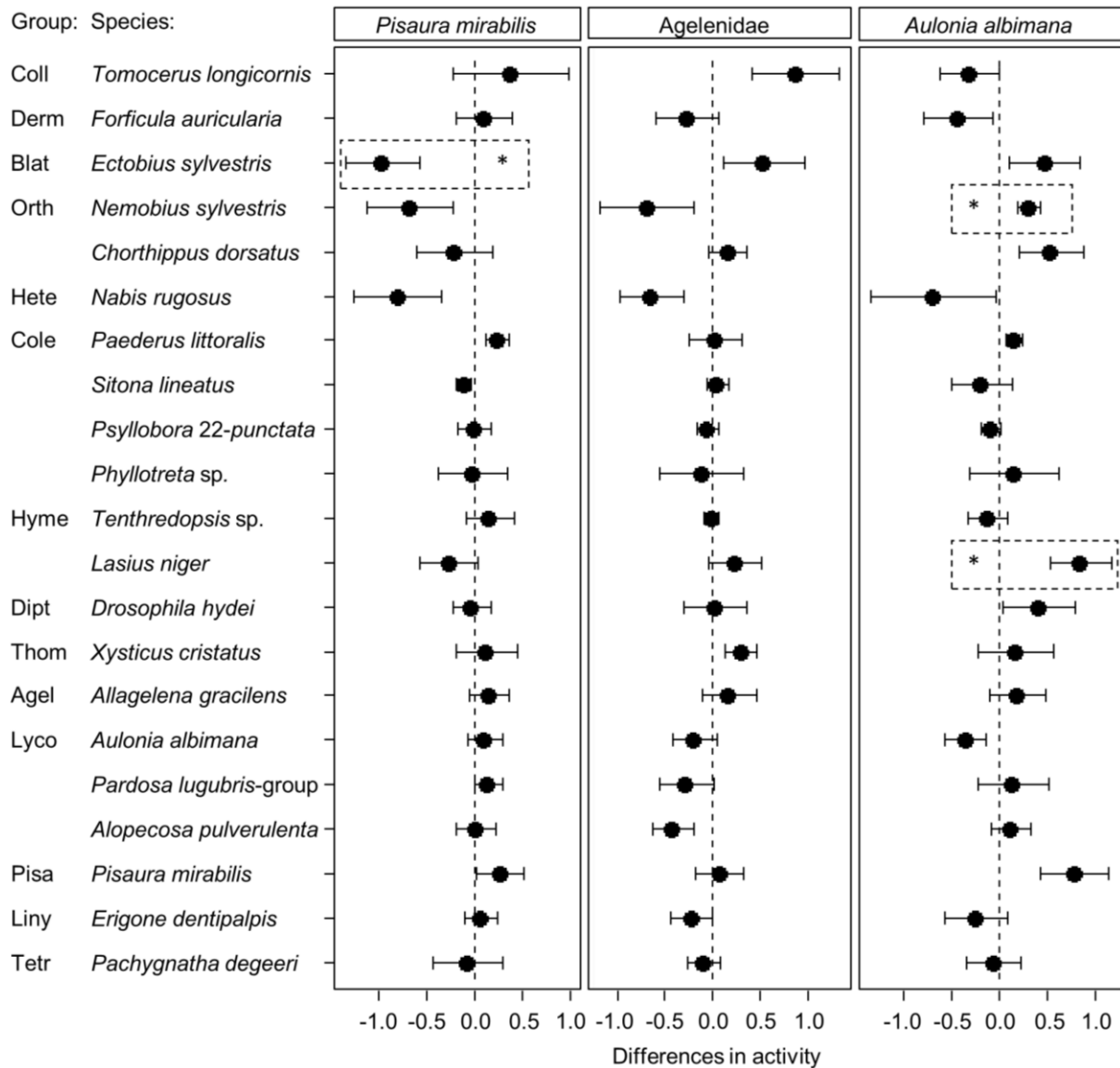
Figure 1.2: Differences in Prey activity between filter paper halves with and without predator chemotactile cues (mean ± se) in the choice set up. Positive values indicate an increase in activity on the predator cue side, negative ones a decrease compared to the cue-less side ($p < 0.01$ **, $p < 0.05$ *). See Fig. 1.1 for group abbreviations.

1.5. Discussion

Rarity of behavioral effects of spider chemotactile cues

Surprisingly, behavioral changes in response to chemotactile cues of three spider species were generally rare among the tested species. Former studies using the same methods reported clear changes in behavior of wolf spiders (*Pardosa milvina*) and crickets (*Gryllus pennsylvanicus*) upon contact with chemotactile cues of the larger wolf spider *Tigrosa helluo* (Persons et al. 2001; Storm and Lima 2008). For example, Persons et al. (2001) found that in *Pardosa* spiders as prey species, behavioral metrics such as time spent immobile differed two-fold between cue-bearing and cue-less filter papers. If the predators were fed with prey of the respective species before the experiment, effect size even doubled. In field crickets (*Gryllus pennsylvanicus*) Storm and Lima (2008) found in average a 1.8-fold increase in time spent immobile in response to cues of three wolf spider species (*Tigrosa helluo*, *Rabidosa rabida*, and *Rabidosa punctulata*). If the predator was fed with field crickets prior to the experiments time spent immobile differed two-fold between treatments. Compared to our no-choice experiment wood crickets (*Nemobius sylvestris*) showed in average a 1.3-fold increase in time spent immobile in response to chemotactile cues of *Allagelena gracilens* and *Pisaura mirabilis*. The number of replicates per combination was restricted by the large number species studied. However, ten replicates per treatment would be sufficient to reveal behavioral effects of previously reported strength.

Due to the 63 species combinations including 1890 tested prey individuals and hundreds of predator individuals for cue deposition we faced several further limitations: It is likely that the response to predator cues varies between female and male prey individuals (Sitvarin & Rypstra 2012). For most insect prey species we were not able to distinguish between sexes (e.g. because immature individuals were used). However, for predators we excluded adult male individuals and used only adult females (Agelenidae and *Aulonia*) or mainly adult females and immature individuals in the case we run out of adult females (*Pisaura*, Table 1.1). Previous consumption of conspecifics can magnify behavioral effects (Murray & Jenkins 1999; Kortet & Hedrick 2004). We demonstrate that for example crickets are capable of detecting spiders also in the absence of conspecific cues, because spiders were kept in the lab for at least one week

prior to the experiment and fed with *Drosophila hydei*. This suggests that the chemotactile cues of the predator alone are sufficient to induce behavioral changes, but diet-based cues may have an additional effect (Persons et al. 2001). In contrast, fruit flies (*Drosophila hydei*) did not respond to cues of three spider species that were fed conspecifics, indicating that diet-based cues alone do not necessarily induce a behavioral response. Alternatively, the lack of syntopic occurrence may explain the absence of behavioral changes of fruit flies towards the tested predators. The remaining 20 prey species overlapped in microhabitat and temporal occurrence with the tested predators. In addition to previous food sources, the hunger level of predators may influence the strength of behavioral response: hungry spiders are expected to induce stronger behavioral response compared to satiated ones (Bell et al. 2006). Here we kept the spiders used for predators on an intermediate hunger level. Thus, the used spiders definitely posed a risk for the tested prey species.

Our results suggest that the ability to recognize chemotactile cues of the three spider species is rare. Alternatively, spider cues may lead to changes in behavior that remain undetected with our methods. Moreover, it has been demonstrated that chemotactile cues of the larger wolf spider *Tigrosa helluo* degrade if filter paper is moistened after cue deposition (Wilder et al. 2005). In contrast to Wilder et al. (2005) we moistened filter papers before cue deposition by spiders to avoid desiccation (see cue preparation). The possibility that cue deposition is impaired on moist filter paper may have contributed to the relatively weak effects observed in the current study. However, natural surfaces at ground level are often moist in the study area, so the moistened filter paper does represent a realistic situation. Former studies that used similar methods and which highlight the importance of antipredator behavior driven by spider cues include organisms that showed particularly strong reactions in our experiments (e.g. crickets: Storm and Lima 2008; Kortet and Hedrick 2004). Possible publication bias towards species combinations that show particular strong behavioral responses may overestimate the occurrence of behavioral effects driven by spider chemotactile cues. The observed rarity of behavioral effects of spider cues may be due to diffuse coevolution between generalist predators and their prey (Caro 2005). Prey species have to evolve antipredator strategies (e.g. increase or decrease of activity)

against various predators and a successful antipredator strategy against a particular predator/cue combination may be maladaptive towards another one. Also similarities in cues of predators that demand different antipredator strategies may weaken coevolutionary forces. In addition to the diffuse selective forces, prey individuals have to balance the risk of predation to the incurred costs (Sih 1980; Lima & Bednekoff 1999; Lind & Cresswell 2005).

Crickets and cockroaches: non-consumptive effects between predator and prey
Wood crickets increased their activity in the presence of spider cues in three independent experiments. It is already known that crickets can detect and memorize cues related to diet and recognition of conspecifics (Matsumoto & Mizunami 2006; Weddle et al. 2013) and that they change their behavior in the presence of spider chemotactile cues (Kortet and Hedrick 2004; Storm and Lima 2008). In contrast to our results, earlier studies found an opposite response: crickets became less active in the presence of spider cues. In accordance with these earlier findings, the cockroach *Ecotobius sylvestris* decreased its activity in the presence of *Pisaur*a cues. Such a "freezing" behavior was interpreted as a strategy to reduce predation risk, because a moving prey is particularly attractive for spiders that are sensitive to movement and vibratory cues, while immobile prey individuals are usually overlooked by spiders (Persons & Uetz 1996; Rovner 1996). However, various spiders scavenge on dead insects including crickets (*Acheta domesticus*) even when live prey is available (Knost & Rovner 1975; Vetter 2011). In such cases, increasing instead of decreasing movement activity is likely to reduce predation risk. Our finding of increased and decreased activity in risk-exposed crickets and cockroaches demonstrates that the antipredator strategy can vary within narrow taxonomic groups. Moreover, the direction of behavioral response in the wood cricket *Nemobius sylvestris* changes during their life cycle: In early summer large adult crickets became less mobile in the presence of chemotactile cues of *Pisaura mirabilis* (Binz et al. 2014), whereas the smaller juveniles used in our experiments became more active.

Unexpectedly, the results even differ between the two experimental setups: In the no-choice experiment crickets responded significantly to chemotactile cues of *Pisaura*

mirabilis and *Allagelena gracilens*, whereas in the choice experiment crickets showed significant behavioral changes only on cues of *Auolonia albimana*. We suggest that these contrasting results may partly be explained by the properties of the chemotactile cues that mediate the behavioral responses. For the two web-building spiders, cricket behavior in the choice arena was more similar to the cue treatment than to the control of the no-choice setup. In contrast, cricket behavior in relation to *Pisaura* cues in the choice experiment was closer to the control of the no-choice setup. We see a clear need to identify relative importance of different cue sources (e.g. feces, chemical footprints, and/or dragline silk) and the detailed chemical properties of the substances that mediate these non-consumptive effects. To date little is known about the substances that are involved in interspecific interactions (Schulz 2004). Compared to chemical profiles of insects, preliminary analysis of spider cuticula revealed an unexpected variety of chemical compounds that complicates their identification (data not shown).

Spiders and ants: non-consumptive effects between predators

Besides the consumption of intraguild prey (i.e. intraguild predation; Polis et al. 1989), top predators can interfere with other predators by changing their behavior (Whitehouse et al. 2011). The increase in activity of *Lasius niger* on *Aulonia albimana* cues was the only consistent effect across both experimental setups. Interestingly the ecological relationship between both species is largely unknown. Early studies mentioned that *Aulonia albimana* is spatially associated with ants in the field (Van der Aart & De Wit 1971), which was explained with the preference for similar vegetation structure, hiding places, and humidity (Boevé 1992). Individuals of *Lasius niger* were not eaten by *Aulonia albimana* even if they were kept together for a week (personal observation). Thus, we suggest that *Lasius niger* increased its activity to avoid intraguild interference with *Aulonia albimana*.

Among spiders, nursery web spiders *Pisaura mirabilis* reduced their movement in response to conspecific chemical cues. Because we used adult female or immature individuals, we exclude a sexually-motivated reaction. This behavioral change occurred only in either of the two experimental setups, which may again point to different properties of the chemotactile cues. The observed decrease in activity may be to avoid

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detection an encounter with other spiders. Although it is not specifically known for *Pisaura mirablis*, encounters between spiders often end up with one being eaten, and competitive interactions are frequent among spiders (Wise 1993). Several studies documented a clear decrease in activity of *Pardosa milvina* in the presence of cues of *Tigrosa helluo* (Persons & Rypstra 2001; Persons et al. 2002). Besides this model system, little is known about antipredator behavior of spiders. In a field experiment, both predation spiders (unmanipulated wolf spiders) and risk spiders (wolf spiders with glued chelicerae) equally reduced densities of sheet web spiders (Linyphiidae) (Schmidt-Entling and Siegenthaler 2009). This study suggests that sheet-web spiders left plots preoccupied by wolf spiders to avoid intraguild interference. Such non-consumptive effects can be of particular importance for ecosystem functioning and pest control because they affect the impact of generalist predators on prey populations (Finke & Denno 2005; Straub et al. 2008).

1.6. Conclusion

Behavioral arena experiments connected with automated video analysis allowed us to screen numerous species combinations for interspecific behavioral responses. We did not find a uniform behavioral response to spider chemotactile cues. Instead, behavioral changes were specific to only few prey species. We suggest that the rarity of behavioral effects of spider cues may be due to diffuse coevolution between generalist predators and their prey. In accordance with former studies our experiments revealed that crickets in particular respond to spider chemotactile cues. Further experiments under more natural conditions should clarify if behavioral effects driven by spider cues enhance prey survival and to which extent they cascade down to lower trophic levels.

1.7. Acknowledgements

2.

Knowing the risk: crickets distinguish between spider predators of different size and commonness

Wood crickets (*Nemobius sylvestris*) performing in the subdivided arena experiment

© Hellena Binz and Roman Bucher

This chapter is based on an original research article published in *Ethology* in January 2014:

2.1. Abstract

Predators unintentionally release chemical and other cues into their environment that can be used by prey to assess predator presence. Prey organisms can therefore perform specific antipredator behavior to reduce predation risk, which can strongly shape the outcome of trophic interactions. In contrast to aquatic systems, studies on cue-driven antipredator behavior in terrestrial arthropods cover only few species to date. Here, we investigated occurrence and strength of antipredator behavior of the wood cricket *Nemobius sylvestris* towards cues of 14 syntopic spider species that are potential predators of wood crickets.

We used two different behavioral arena experiments to investigate the influence of predator cues on wood cricket mobility. We further tested if changes in wood cricket mobility can be explained by five predator-specific traits: hunting mode, commonness, diurnal activity, predator-prey body-size ratio, and predator-prey life stage differences. Crickets were singly recorded (1) in separate arenas, either in presence or absence of spider cues, to analyze changes in mobility on filter paper covered with cues compared to normal mobility on filter paper without cues, and (2) in subdivided arenas partly covered with spider cues, where the crickets could choose between cue-bearing and cue-less areas to analyze differences in residence time and mobility when crickets are able to avoid cues.

Crickets either increased or reduced their mobility in the presence of spider cues. In the experiments with cues and controls in separate arenas, the magnitude of behavioral change increased significantly with increasing predator-prey body-size ratio. When crickets could choose between spider cues and control, their mobility was significantly higher in the presence of cues from common spider species than from rare

spiders. We therefore conclude that wood crickets distinguish between cues from different predator species and that spiders unintentionally release a species-specific composition and size-dependent quantity of cues, which lead to distinct antipredator behavior in wood crickets.

2.2. Introduction

Prey organisms can change their behavior upon the detection of predators or the cues they release unintentionally. These changes in turn can affect mating, feeding, movement and ultimately survival of prey individuals (Persons et al. 2002; Werner & Peacor 2003; Preisser et al. 2005). By cascading down to lower trophic levels (e.g. herbivores; Martinou et al. 2009, Schmidt-Entling & Siegenthaler 2009), these non-consumptive effects can affect ecosystem functions like plant productivity, energy flux and nutrient cycling (Schmitz et al. 2008; Hawlena et al. 2012). For example, predator cues from green crabs (*Carcinus maenas*) suppress grazing of herbivorous snails (*Littorina littorea*), which in turn decreases snail growth and increases abundance of fucoid algae (Trussel et al. 2002). As such non-consumptive effects can strongly influence community dynamics, their interest to ecologists has increased rapidly in recent years (Preisser & Bolnick 2008b; Preisser et al. 2009; Jones & Dornhaus 2011).

According to Helfman's threat-sensitive avoidance hypothesis (1989) and Lima and Bednekoff's predation risk allocation hypothesis (1999), prey individuals should adjust their antipredator behavior to the degree and type of predation threat they currently experience (Persons et al. 2001; Hoefler et al. 2012). Therefore, antipredator behaviors can vary strongly in strength and direction, which again influences the number of implemented defense types (Kats & Dill 1998; Caro 2005). Bank voles for example increased the number of antipredator responses depending on the degree to which a predator specialized on them. They displayed four different antipredator behaviors in response to odors of a specialized predator (weasels) but only two in response to cues of a generalized one (red fox; Jędrzejewski et al. 1993).

Species-specific traits of predator and prey strongly influence the conditions under which prey individuals show antipredator behavior, including body size, diurnal activity, and commonness. The strength of antipredator behavior is for example linked

with size ratio of predator and prey. Size ratio is known to limit a predators' potential to subdue prey (Warren & Lawton 1987; Brose et al. 2006; Digel et al. 2011). Moreover, a relatively large prey can pose a substantial threat to the predator itself (Gabbutt 1959; Hayward et al. 2006; Whitman & Vincent 2008; Mukherjee & Heithaus 2013), while very small prey may be ignored, as the net energetic gain for the predator is too low (Stephens & Krebs 1986; Provost et al. 2006). Antipredator behavior therefore varies directly with strength of predation risk, as assessed by the predator:prey size ratio (Helfman 1989; Persons & Rypstra 2001; MacNulty et al. 2009).

Predator hunting mode also influences prey antipredator behavior. In contrast to active predators, cues of sedentary predators, like sit-and-wait or sit-and-pursuit hunters, reliably predict their nearby presence or their presence in the immediate past (Kats & Dill 1998; Schmitz & Suttle 2001; Preisser et al. 2007; Miller et al. 2013). This prediction should be even more reliable for predators that deploy silk in prey capture. As silk alone can also induce antipredator behavior (Rypstra & Buddle 2013), predators that use silk in prey capture may release a higher total amount of potential chemotactile cues on one spot (in addition to e.g. feces and chemical footprints) than those who do not. Being able to recognize cues of predictable predators allows prey individuals to lower their predation risk, whereas recognizing cues of unpredictable active predators is less informative and may therefore have a less pronounced effect on prey behavior.

A similar differentiation should arise for cues of common compared to rare predator species. According to the 'rare enemy effect', prey should not evolve costly avoidance tactics to rare predators that bear low predation risk (Dawkins 1999). Consequently, the strength of antipredator behavior should increase with predator commonness.

Moreover, the diurnal activity cycle of a predator can also influence antipredator behavior, since nocturnal predators use different stimuli for prey detection than diurnal ones. While nocturnal predators mainly depend on vibratory stimuli for locating and capturing prey (Uetz & Roberts 2002; Young & Morain 2002; Foelix 2011), diurnal predators also hunt by sight (Persons & Uetz 1997; Archer et al. 1999; Clemente et al. 2010). Prey are therefore expected to decrease mobility when detecting cues of nocturnal predators, as immobility reduces potential vibratory prey cues. In contrast,

when facing cues of diurnal predators, increased mobility may be advantageous in order to increase distance to a potential predator, especially when the prey is not well camouflaged (Lima and Dill 1990).

Interactions between predator and prey species also depend on age-related sizes as assemblages of opponents changes during growth. First, predators' size during different life stages determines the range of manageable prey and therefore predators' hunting strategies (Caro 2005; Radford 2008; Krala & Poteserb 2009). Second, younger prey is less able to defend themselves because of their small size which can lead to changes in antipredator behavior during ontogeny (Creer 2005; Dangles et al. 2007). Relating antipredator behavior to life-history traits of the predators could therefore lead to a more general understanding of their role in natural food webs (Werner & Peacor 2003; Schmitz et al. 2004; Preisser et al. 2005).

The aim of this study was to determine the occurrence and strength of antipredator behaviors of wood crickets (*Nemobius sylvestris*) in response to a wide taxonomic range of spiders as their potential predators. Crickets occur in many habitat types and serve as prey for a wide range of different spider species (Gabbutt 1959; Detzel 1998). They have recently been shown to respond to the chemotactile cues of spiders (Kortet & Hedrick 2004; Storm & Lima 2008). Most studies have focused on the reactions of a few prey organisms such as wolf spiders and crickets towards a limited number of predatory spider species (Persons & Rypstra 2001; Persons et al. 2001; Kortet & Hedrick 2004; Storm & Lima 2008, 2010). A comparison across broader ranges of predatory spider taxa allows drawing general conclusions on the commonness of crickets' antipredator behavior and on the influence of certain life-history traits of spiders on cricket antipredator behavior.

We conducted two different experiments in the lab to determine effects of predator identity on the magnitude of antipredator behavior: (1) 'Separate Arena' experiment: When facing cues from predators of different risk intensities, prey should reveal qualitative differences in antipredator behaviors (e.g. Jędrzejewski et al. 1993). We analyzed behavioral changes in wood crickets when facing chemical cues from 14 different spider species compared to the normal behavior in a control arena without cues. (2) 'Subdivided Arena ' experiment: When arenas contain areas with and without

predator cues, prey should avoid or behave differently in cue-bearing areas (Ivins & Smith 1983; Petranka et al. 1987; Barreto & Macdonald 1999; Séquin et al. 2003).We tested wood crickets' mobility and time spent on cues between cues from 14 different spider species and the respective controls in the same arena. We expected wood cricket antipredator behavior to differ between experimental set-ups because of different opportunities to avoid predator cues. In the 'Subdivided Arena' experiment, wood crickets had the possibility to leave the side with predator cues, giving them resting and safety opportunities which was completely lacking in the 'Separate Arena' experiment, where wood crickets were put under seemingly constant predation pressure with no opportunity to escape.

In our study system we hypothesized and tested whether antipredator behavior depended on the following predator traits:

1. *Predator hunting mode*: Antipredator behavior should be stronger against cues from sit-and-wait predators than against actively-hunting predators (Miller et al. (2013). We therefore compared reactions towards cues of five actively hunting species to those of three sit-and-pursue , two sit-and-wait and four web-building species, respectively (classified in table 2.1 according to Roberts 1996).

2. *Predator commonness*: Rare species should evoke less antipredator behavior than common species (Dawkins 1999). We therefore analyzed antipredator behavior as a function of predator commonness (as determined by Hänggi et al. 1995).

3. *Predator diurnal activity*: Cues from nocturnal spiders are predicted to lower wood cricket mobility whereas cues of diurnal spiders should increase their mobility. We compared antipredator behavior towards cues of three nocturnal vs. eleven diurnal spider species.

4. *Body size ratio of wood cricket vs. its predator*: Predator:prey body size ratio should be positively correlated with antipredator behavior (Persons & Rypstra 2001). We tested this hypothesis across our 14 species.

5. *Life stage of spider and wood cricket*: *Pisaura mirabilis* exhibits two different hunting modes during its life cycle (immature: web-building, adult: sit-and-pursuit,

Lenler-Eriksen 1969; Roberts 1996). As cues of immatures should be better predictable (see hypothesis 1) and cues of adults should be of a higher quantity (see hypothesis 4) we expected wood cricket antipredator behavior to be equally strong towards both life stages of *P. mirabilis*. Wood crickets on the other hand show different escape performances during their ontogeny (immature: fast escape, adult: slow escape, Gabbutt 1959; Dangles et al. 2007). When facing cues of *P. mirabilis*, young wood crickets should therefore increase mobility more than adults. We compared antipredator behavior of adult wood crickets in the presence of adult *P. mirabilis* versus antipredator behavior of adult and immature wood crickets to immature *P. mirabilis* in the same experimental set-up.

2.3. Methods

Collection and keeping of study species

The flightless ground-dwelling cricket species *Nemobius sylvestris* has a reduced dispersal ability (Brouwers et al. 2011), but is widespread in Europe with a strong association with woodland habitat (Brouwers & Newton 2009, 2010). In the study region, populations occur in mesic to dry forest stands, often close to south-exposed forest edges (Detzel 1998). As predators, we used 14 spider species as taxonomically diverse, and with as different species-specific traits as possible (Table 2.1, Hänggi 1995; Roberts 1996). All species prey on wood crickets (HB pers. obs.) and co-occur with them on the collection sites. To minimize phylogenetic effects, we chose spider species from 12 different families. Only for Lycosid spiders, three species were included, which differed in hunting mode, body size, and abundance (Table 2.1). Because of problems in collecting nocturnal spider species, we only could include three in this study, compared to eleven diurnals. Spider species and crickets were captured between March and June 2011 (except *Pisaura mirabilis* and young crickets, which were captured until mid-September 2011) at two sites in the vicinity of Landau (Rhineland-Palatine, Germany). The first site (Bellheim: 49°11'45" N / 8°19'11" E) was a heath-like open vegetation within a mixed forest stand and contained all

Table 2.1: Spider species used in this study. Hunting mode and activity time according to Roberts (1996). Commonness is given as the number of dry deciduous forest, pine forest and dry heathland sites (54 sites in total) where a species was recorded in Hänggi et al. 1995, which covers habitats across central Europe (A. Hänggi, unpublished data and Hänggi et al 1995). Asterisk indicates the most common species represented in the study and in the investigated area.

Spider family	Spider species	Spider code	Hunting mode	Common-ness	Activity time
Agelenidae	*Agelena labyrinthica*	Al	Web	6	Day
Amaurobiiade	*Amaurobius fenestralis*	Af	Web	6	Night
Dysderidae	*Dysdera erythrina*	De	Active	2	Night
Gnaphosidae	*Drassodes cupreus*	Dc	Active	0	Night
Lycosidae	*Aulonia albimana*	Aa	Web	6	Day
	Alopecosa pulverulenta	Ap	Sit-and-pursue	10	Day
	Pardosa lugubris gr.	Pl	Active	36*	Day
Oxyopidae	*Oxyopes ramosus*	Or	Sit-and-pursue	1	Day
Philodromidae	*Tibellus oblongus*	To	Sit-and-wait	2	Day
Pisauridae	*Pisaura mirabilis*	Ad Pm Im Pm	Sit-and-pursue	8	Day
Tetragnathidae	*Pachygnatha degeeri*	Pd	Active	8	Day
Theridiidae	*Enoplognatha ovata*	Eo	Web	9	Day
Thomisidae	*Xysticus cristatus*	Xc	Sit-and-wait	19	Day
Zoridae	*Zora spinimana*	Zs	Active	18	Day

studied species. All crickets and most spider individuals were obtained from this site. This ensured syntopic occurrence, which is relevant for the evolution and/or ontogenetic development of antipredator behavior. The second site (Gleishorbach: 49°07'25" N / 7°59'34" E) was a heterogenic fallow-like pasture where additional individuals of the studied spiders were obtained. Live individuals were collected with suction sampling, pooter and pitfall traps. Individuals were maintained singly in 40 ml glasses with moistened plaster of paris as ground in a climatic chamber (16 °C, 65% humidity and natural day/night rhythm). They were fed to satiation with *Drosophila hydei* over the whole study period. Crickets were kept together in a plastic terrarium

(30x20x20 cm) with a ground layer of plaster of paris, which was periodically moistened and fed with apple and carrots *ad libitum*. Before each experiment we measured (1) weight and (2) body width (prosoma in spiders, thorax in insects) for each spider and cricket individual (Appendix A2.1). At the end of the experiments at least one voucher specimen of each species was stored in alcohol whereas the rest of the arthropods were released in their original habitats.

Experimental design

We tested the influence of predator identity on prey antipredator behavior by confronting wood crickets with cues from 14 different spider species (Table 2.1) in comparison to cue-free controls. Furthermore, we tested the reaction of wood crickets towards conspecific cues. We did not study cues of further non-predatory species since we were interested in the reaction towards different predator species, not in a general differentiation of predatory and non-predatory cues. Moreover, all spider species of sufficient size could represent potential predators, and, as insects are likely to leave cues that differ from spider cues chemically and physically (Trabalon & Bagnères 2010; Goller 2008), we did not test cues from further non-predatory species. Each predator-prey combination and the conspecific combination were tested 10 times per treatment between mid-May and July 2011 with different individuals of the respective species, resulting in ten trials per combination. Two different designs were used: in the 'Separate Arena' experiment, cricket individuals in arenas with or without predator cues were compared (design borrowed from Storm & Lima 2008, 2010). In the 'Subdivided Arena' experiment, each arena consisted of an area with predator cues and an area without (design borrowed from Persons & Rypstra 2001). For the 'Separate Arena' experiment we lined experimental arenas with filter papers and performed 10 replications each with and without spider cues per spider-cricket combination (n = 14 × 20 = 280 trials). Arenas consisted of a reusable plastic tube with a diameter of 10 cm at the base and 12 cm at the top (15 cm height) and a Petri dish serving as exchangeable arena floor. Since the crickets measured 9.03 +/- 1.74 mm SD, this size was sufficient to reliably record short-term mobility metrics. Predator cues were obtained by keeping spiders on the moistened filter papers in a climate cabinet (25°C and 75 % humidity)

for 24 hours prior to the experiment. The cues for each trial stemmed from a single spider individual; and each of the ten spiders per species were only used for one trial. Control filter papers were treated the same way, but without spider contact. After removing the spiders, each of the filter papers were placed on a new Petri dish at the bottom of the arenas. Individuals of *N. sylvestris* were then placed into the centers of the arenas and five arenas, each with one cricket, were simultaneously filmed for 15 minutes with a video camera (SONY HDR-CX 550 VE) mounted on a tripod. Former studies used longer filming periods (e.g. 20 min: Storm & Lima 2008, 2010 ; 1 h: Persons & Rypstra 2001) but as we observed no further movements of wood crickets after 15 minutes, we decided to shorten filming periods according to this. In the 'Subdivided Arena' experiment 10 experimental arenas were covered with two semi-circles of filter paper, one containing predator cues and one serving as control. As we used semi-circles of filter paper, one of the five spider individuals per species provided cues for two trials. The filming was performed as in the 'Separate Arena' experiment, but as for each of three predator species (*Amaurobius fenestralis*, *Agelena labyrinthica* and *Xysticus cristatus*) one cricket did not move at all during the 15 minutes, those trials were excluded from the analysis (i.e. n = 9 instead of n = 10 trials for these three predator species; total n = 137 trials for the 'Subdivided Arena' experiment).

The combination *Pisaura mirabilis* - *N. sylvestris* revealed particularly strong antipredator behavior and we decided to repeat the 'Separate Arena' experiment to compare between life stages (Experimental times: mid-July and mid-September 2011).

Video analysis and statistics

We used the tracking software EthoVision XT8 (Noldus Information Technology, Wageningen, The Netherlands) to automatically analyze mobility and residence time on filter paper and the respective zones of *N. sylvestris* during the time of the experiment with 5 frames per second. The mobility was determined by a mobility threshold of 5% pixel changes per frame of the complete area detected as cricket. This means, that wood crickets were recorded as immobile, as long as pixels surrounding the wood cricket body changed less than 5 % per frame. Only by exceeding the threshold of 5 %, wood crickets were recorded as mobile. Relative time mobile on the

respective filter paper served as measure for cricket mobility. For further analysis, we subtracted mobility on cue-less control filter papers from those of the cue-bearing ones (Subdivided Arena experiment: paired data, Separate Arena experiment: median of the control mobility per spider-cricket combination was calculated and then subtracted; although control mobility was normally distributed, median was used to balance marginal intracombinational differences in the variance of the control mobility), resulting in positive (more mobile in the presence of cues) and negative Δ mobility values (more immobile in the presence of cues).

Using one sample *t* tests (mu=0), we compared an overall difference in cricket mobility (for 'Subdivided Arena' and for 'Separate Arena' experiment) and cricket residence time (for 'Subdivided Arena' experiment) between control and cue filter papers, pooled for all spider species. For the Subdivided Arena experiment, each spider individual was used for two filter paper halves; in these *t* tests, we therefore calculated the averages of the two assays for each spider individual that had provided cues, resulting in n = 70 for all spiders. For both experimental setups, the Δ mobility values of wood crickets towards each spider species were tested against an intercept of zero with a linear model (hence, df = 14) and were then Bonferroni-corrected for multiple comparisons.

For analyzing the influence of the different life stages in the combination *Pisaura mirabilis - N. sylvestris*, we also tested Δ mobility against zero for each life stage combination. Since we noticed that the magnitude of mobility change varied, we additionally analyzed the magnitude of mobility change (|Δ| mobility) as a dependent variable.

For both setups separately, we then used linear regression models to test for the effects of hunting mode (web, sit-and-wait, sit-and-pursue and active), activity time (diurnal or nocturnal), commonness in the study habitat (i.e. number of sites of dry deciduous forest, pine forest and dry heathland habitats where a species occurred; based on raw data kindly provided by Ambros Hänggi (Hänggi et al. 1995)) and spider size (prosoma width and body weight for each spider species) and predator:prey size ratio (width, and weight ratio per spider:wood cricket combination) on Δ and |Δ| mobility. Each significant predictor was then tested using a linear mixed-effect model

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fitted by residual maximum likelihood (REML) or analysis of deviance tables (Anova, type 2 tests, package 'car'), each with predator species as random factor.

Beside the spider-cricket assays, we analyzed cricket behavior (Δ mobility) towards cues of conspecifics for comparison (*t* tests, Separated Arenas: n = 10; Subdivided Arenas: n = 5 cue providing individuals, n = 10 assays). All statistical analyzes were calculated in R 2.15.1(R Development Core Team 2013)using the packages MASS (Venables & Ripley 2002), nlme (Pinheiro et al. 2012), sciplot (Morales & R Development Core Team 2012)and car (Fox et al. 2012).

2.4. Results

1.1 'Separate Arena' experiment

In the presence of spider cues (pooled for all species), wood cricket mobility neither generally increased nor decreased (*t* test: t_{139} = 0.14, p = 0.89). However, mobility varied with spider species (LM: R^2 = 0.28, $F_{14,126}$ = 4.30, p < 0.001, Fig. 2.1). Compared to control arenas, wood crickets showed a higher mobility in the presence of cues from *Agelena labyrinthica* (t_9 =3.19, p = 0.002) and *Drassodes cupreus* (t_9 =3.05, p = 0.003), whereas they significantly reduced their mobility in the presence of cues from *P. mirabilis* (t_9 =-3.01, p = 0.003; all three effects remain significant after Bonferroni correction). All other spider species did not induce a significant change in mobility.

Across different spider species, wood cricket antipredator behavior significantly depended on spider:cricket size ratio. The absolute change in wood cricket mobility (|Δ| mobility) significantly increased with increasing spider:cricket weight ratio (LME: R^2 = 0.68, t_{125} = 3.59, p < 0.001, in Fig 2.2 mean values per spider:cricket ratio). The same relationship held for spider weight, width, and width ratio instead of weight ratio (all p < 0.001). A similar relation was also found when only considering the increase (LME: R^2 = 0.68, t_{62} =2.10, p = 0.039; *n = 7* species; black circles in Fig.2.2), but not the decrease in mobility (LME: R^2 = 0.72, t_{62} =-1.35, p = 0.18; n = 7 species, open circles in Fig. 2.2). Spider hunting mode, commonness and activity time had no influence on wood cricket behavior (all p > 0.46). Wood crickets showed no significant behavioral reaction towards cues from conspecifics compared to cue-free filter paper (*t* test: t_9 = 1.29, p = 0.23).
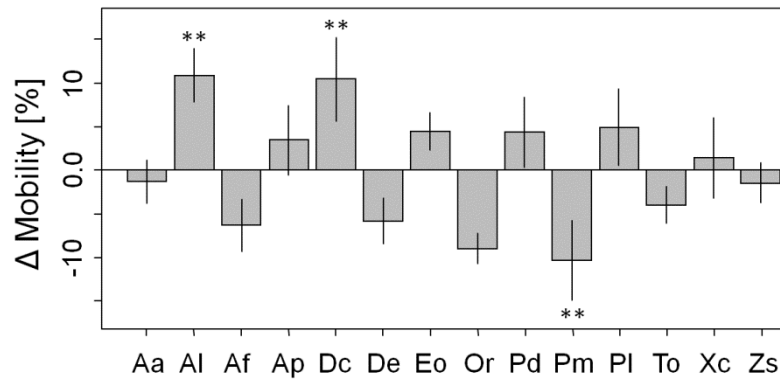
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Figure 2.1: 'Separate Arena' experiment: Comparison of mobility changes in the wood cricket *Nemobius sylvestris* in the presence of cues from different spider species. Positive values indicate higher, negative values lower wood cricket mobility on filter paper covered with spider cues compared to the control (0). Significance levels are based on linear models: **p < 0.01. Spider species codes: Aa = *Aulonia albimana*, Al = *Agelena labyrinthica,* Af = *Amaurobius fenestralis*, Ap = *Alopecosa pulverulenta*, De = *Dysdera erythrina*, Dc = *Drassodes cupreus*, Eo = *Enoplognatha ovata*, Or = *Oxyopes ramosus*, Pd = *Pachygnatha degeeri*, Pl = *Pardosa lugubris-group*, Pm = *Pisaura mirabilis*, To = *Tibellus oblongus*, Xc = *Xysticus cristatus*, Zs = *Zora spinimana*. *N* = 20 (10 control + 10 cue covered filter papers) per combination.
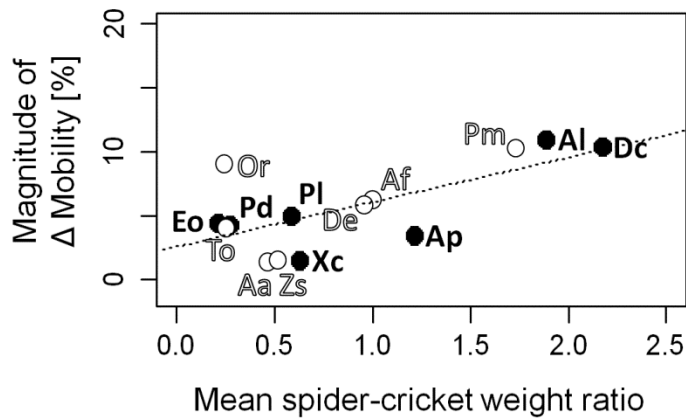

Figure 2.2: 'Separate Arena' experiment: Magnitude of mobility change in the wood cricket *Nemobius sylvestris* vs. mean spider:cricket width ratio. Black dots indicate higher, open dots lower mobility of wood crickets on filter paper with spider cues than on the control. Spider species codes as for Fig. 2.1. *N* = 20 (10 control + 10 cue-bearing filter papers) per combination. **significantly different from zero at p < 0.004

1.2 Influence of life stages

In addition to the adult life stage combination of *P. mirabilis* - *N. sylvestris* (see above), we tested the effect of cues from immature *P. mirabilis* on adult and immature wood crickets. The response of wood crickets to spider cues differed between life stages of predator and prey (LM: R^2 = 0.48, $F_{3,27}$ = 8.36, P< 0.001, Fig. 2.3). When predator and prey were immature, crickets increased their mobility (t_9 = 3.34, P = 0.005). However, immature *P. mirabilis* did not affect the mobility of adult wood crickets (t_9 = 0.55, p = 0.59). Finally, when both, spiders and crickets were adult a decrease in cricket mobility was observed (t_9= -3.92, p < 0.001). The absolute change in wood cricket mobility (|Δ| mobility) was similar for both cricket life stages (*t* test: t_{18} = 0.83, p = 0.42).
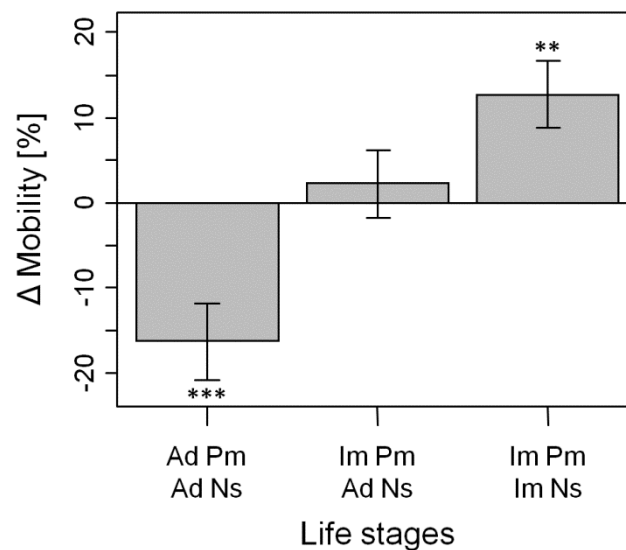

Figure 2.3: 'Separate Arena' experiment: Comparison of mobility changes in immature (Im) and adult (Ad) wood crickets (*Nemobius sylvestris*, Ns) towards cues of immature or adult *Pisaura mirabilis* (Im and Ad Pm). Positive values indicate higher, negative values lower mobility on spider cues compared to the control (0). Significance levels are based on linear models: ***p < 0.001, **p < 0.01. N = 20 (10 control + 10 cue-bearing filter papers) per combination.

2.1 'Subdivided Arena' experiment

If wood crickets were given a choice between filter paper halves with and without spider cues (pooled for all species), they showed no overall difference in residence time (*t* test: t_{69} = -1.04, p = 0.30) but a trend of higher mobility on the filter papers with spider cues (*t* test: t_{68}= 2.20, p = 0.05). The wood crickets' antipredator behavior differed between spider species (LM: R^2 = 0.21, $F_{14,123}$ = 2.38, p = 0.006, Fig. 2.4) although, after Bonferroni correction, no species-specific effects remained significant. Across all

species, wood cricket mobility significantly increased with spider commonness (LME: χ^2= 4.4, df = 1, P= 0.036, Fig. 2.5), and was marginally higher for diurnal compared to nocturnal spiders (LME: χ^2 = 3.0, df = 1, p = 0.085). Spider hunting mode, size and size ratio had no influence on wood cricket behavior (all p > 0.26). As in the 'Subdivided Arena' experiments, wood crickets showed no significant reaction towards cues from conspecifics (t test: t_4 = 0.55, p = 0.61) and no differences in residence time (t test: t_4 = 0.38, p = 0.73).
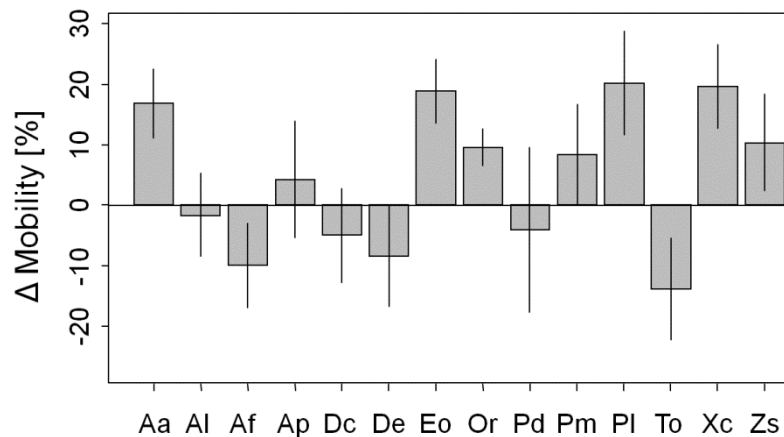

Figure 2.4: 'Subdivided Arena' experiment: Comparison of mobility changes in the wood cricket *Nemobius sylvestris* in the presence of cues from different spider species. Positive values indicate higher, negative values lower wood cricket mobility on filter paper with spider cues compared to the control (0). Spider species codes as for Fig. 2.1. *N* = 10 (half filter paper covered with and half without spider cues) per combination.

2.5. Discussion

Wood crickets perform predator-specific antipredator behavior

Wood crickets showed two responses after detecting cues of distinct spider species: increase and decrease of mobility. The direction of antipredator behavior strongly depended on the spider species. Wood crickets increased their mobility in the presence of cues from *A. labyrinthica* and *D. cupreus*, whereas cues from *P. mirabilis* led to a reduction in mobility in wood crickets. The ability to discriminate at least between predatory spider species and conspecifics has already been shown for the wolf spider *Pardosa milvina*. This prey species did not change its behavior in the
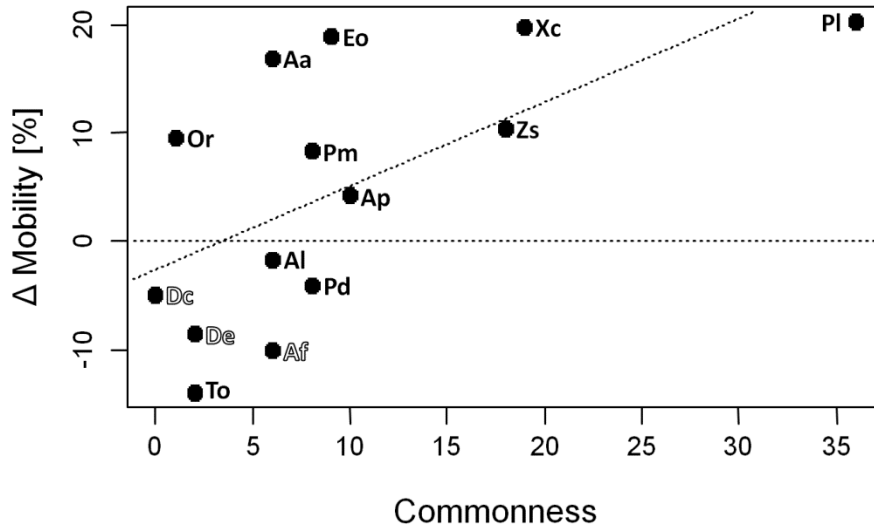
Figure 2.5: 'Subdivided Arena' experiment: Mean mobility of the wood cricket *Nemobius sylvestris* in the 'Subdivided Arena' setup vs. commonness of each spider species in the investigated habitat. Positive values indicate higher, negative values lower wood cricket mobility on filter paper halves with spider cues compared to the control halves (0). Nocturnal spider species are written in white. Spider species codes as for Fig. 2.1. *N* = 10 (half filter paper covered with and half without spider cues) per combination.

presence of chemotactile cues of its conspecifics but became less mobile in the presence of cues from its predator *Hogna helluo* (Araneae, Lycosidae) and responded appropriately to the degree of predation risk (Persons & Rypstra 2001). For avian and mammalian prey species, qualitatively different responses to various predators are well known and differ according to the threat a predator poses (Caro 2005). However, our study is the first to show that a single arthropod prey species (*Nemobius sylvestris*) can react to predatory cues of different species with either an increase or a decrease in mobility. Moreover, opposing changes in mobility were demonstrated for the same species pair (*Pisaura mirabilis /N. sylvestris*), but at different developmental stages. Former studies on crickets and spiders concerning antipredator behavior always found a decrease in prey mobility after detecting predator cues. This may be mainly due to the fact that they exclusively used nocturnal predators (wolf spider *Hogna helluo* as predator of the wolf spider *Pardosa milvina*, (Persons et al. 2001, 2002; Marshall et al. 2002); wolf spider *Hogna helluo* as predator of the field cricket *Gryllus pennsilvanicus*, Storm & Lima 2008, 2010). Nocturnal spiders rely on prey vibrations as attack stimuli, which may favor selection for lower mobility in prey organisms. Wood crickets, in contrast, are mainly diurnal. In both day and night, a decrease of mobility may have the

benefit to decrease predator encounter rates and remain undetected (Werner & Anholt 1993). However, many diurnal spider species can also detect prey visually over larger distances (Persons & Uetz 1997; Clemente et al. 2010). Thus, during the day, increased mobility might likewise be an effective antipredator response, as predators need to invest high energetic costs when following prey over larger distances(e.g. Nordlund & Morrison 1990) and prey avoid being followed by elicit pursuit-deterrent signals (e.g. repeated movements, Hasson 1991; Caro 2005). Our results assume that crickets can adjust their behavior towards different predators, and become either more or less mobile in the presence of cues. However, at the moment we cannot find a clear predictor for this antipredator behavior. In our study, hunting mode did not directly influence the direction of mobility change across all 14 species. Previous studies (Miller et al. 2013, Schmitz & Suttle 2001, Schmitz 2008) found a direct influence of spider hunting mode on grasshopper antipredator behavior. However, these previous studies tested a maximum of two congeneric species per hunting mode. Thus, it remains questionable if spider hunting mode has consistent effects across different spider genus and families. Our results suggest that this is not the case. Instead, the strength and direction of antipredator behavior towards spider species may be linked to multiple life-history traits or combinations thereof. Such traits might e.g. include the sensory channel spiders use to detect prey (vibrational vs. visual; e.g. Lizotte & Rovner 1988; Uetz & Roberts 2002), their use of silk to capture prey (which may differ across, but also within hunting modes, e.g. different web types) their running speed compared to prey speed (Lima & Dill 1990) or even intraspecific variation within these traits (Pruitt et al. 2012). However, as direction of antipredator behavior differed between spiders, we assume spiders to unintentionally release a species-specific composition of cues Relevant cues may be one or several of the following: silk, feces and footprints. Substances of foot prints are chemically identical to the substances on the cuticle surface (Geiselhardt et al. 2009). In contrast to most insects, spider cuticles contain a wide variety of substance classes. Those include hydrocarbons, esters, acids, and alcohols (Goller 2008; Trabalon & Bagnères 2010). Thus, depending on the substance class, prey species may be more or less able to perceive the predator cues, the more so as different substance classes strongly differ in their volatility.

Antipredator behavior depends on predator size

The strength of wood cricket antipredator behavior was positively linked to spider size and to spider:cricket size ratio. This coincides with the expectation that larger spiders release greater amounts of (e.g. chemotactile or volatile) cues than smaller ones (Persons & Rypstra 2001), enabling prey to estimate spider size. Body size limits a predator's capacity to subdue prey and is of major importance in predator - prey interactions. By assessing the predators' size through the amount of emitted cues, prey species could save energy by adjusting their antipredator behavior to the actual predation risk (Persons & Rypstra 2001). Moreover, wood crickets additionally took into account their own size as shown by our findings of distinct cricket antipredator behavior on *P. mirabilis* during different life stages. Immature *P. mirabilis* can easily prey on immature crickets, but not on adult ones (author's pers.obs.). Indeed, immature crickets showed antipredator behavior to cues of immature *P. mirabilis*, but adult crickets did not. Adult crickets, however, did show antipredator behavior in the presence of cues of adult *P. mirabilis.* This reinforces the suggestion that wood crickets evaluate their body size relative to predator size based on the cues they perceive. Interestingly, antipredator behavior of immature and adult crickets did not differ in the magnitude of performance as predicted but differed significantly in its direction: immature crickets were significantly *more mobile* in the presence of immature *P. mirabilis* cues, whereas adult crickets were significantly *more immobile* in the presence of adult *P. mirabilis* cues. But, as expected, strength of behavioral change was similar towards both life stages of *P. mirabilis*. Note that our analysis corrects for mobility on cue-free control areas; hence it is independent of any mobility differences between wood cricket life stages. The presence of two opposite responses in the same predator-prey combinations has, to our knowledge, not been reported before. Although we could not find an effect of hunting mode over the whole set of investigated spider species, wood crickets seemed to distinguish between distinct hunting modes within one species. Young *P. mirabilis* build sheet-like webs with vertical threads, comparable to those of funnel-web spiders (Lenler-Eriksen 1969). The web works as an extension of the legs and aids in prey detection via vibrational signals. Young wood crickets have

good escape ability and could therefore increase the distance to the predator by a fast escape. Adult *P. mirabilis* in contrast are sit-and-pursuit hunters, waiting for its prey to pass before they attack. Adult wood crickets, which can't escape that fast, may therefore benefit from being more immobile around cues of adult *P. mirabilis*.

Antipredator behavior depends on predator commonness

In the 'Subdivided Arena' experiments, wood crickets showed a trend of increased mobility in the presence of spider cues. This performance can be explained by our findings of marginally influence of day/night activity of the respective spiders. Wood crickets were predicted to be more mobile towards cues of diurnal spiders, which mainly hunt by vision, whereas nocturnal spiders did not induce antipredator behaviors. In contrast to the 'Separate Arena' experiment, antipredator behavior did not depend on predator size but on spider commonness. Cricket mobility strongly increased with predator commonness as measured by number of spider records in the investigated habitat types, and rare spiders did not induce behavioral changes. This finding is in accordance with the "rare enemy effect" (Dawkins 1999), which states that prey should only evolve costly avoidance tactics against common predators that exert a high predation risk. Wood crickets live in small local populations and are strongly associated with woodland habitat (Brouwers & Newton 2010) with reduced dispersal ability beyond the forest borders (Brouwers et al. 2011). Therefore, they should experience strong selection pressure by the predator community of this habitat type, which we approximated with the Hänggi commonness data. Alternatively, or in addition to local predator adaptation, olfactory learning and implementing could play a role in wood cricket antipredator behavior. (Matsumoto & Mizunami 2002, 2005, 2006) showed an excellent capacity for olfactory learning in the field cricket *Gryllus bimaculatus*. First, they found out that nymphs of *G. bimaculatus* are able to retain olfactory memory for its lifetime and that it was able to memorize up to seven odor pairs at the same time and associate odors with positive or negative stimuli. Similarly, wood crickets might effectively learn, implement and react to cues of commonly encountered predators, whereas cues of rarely encountered ones would remain unknown to the cricket. As the wood crickets investigated in this study were kept in the

laboratory for a relatively short period of their lifetime (max. 5 month out of 24 month of total lifetime), olfactory memory of wood crickets might be an important factor in explaining wood cricket antipredator behaviors shown herein.

Reduced capture efficiency of common predator species due to widespread antipredator behavior could represent an evolutionary advantage for rare predators, which may contribute to the maintenance of a high diversity of generalist predators (Dawkins 1999). In any case, to what extent selection pressure and/or olfactory learning play a role in evolution and performance of wood cricket antipredator behavior remains open for further study.

Previous studies often used either 'Subdivided Arena' or 'Separate Arena' experiments, but not both (e.g. Storm & Lima 2008, 2010; Persons & Rypstra 2001). In the present study, we used both setups in parallel, and detected different behavioral changes in crickets to spider cues. These differences may be due to the fact that they provide different behavioral options for the cricket. Moreover, the nature of the cues may have an influence: if the cues are highly volatile, they will quickly saturate the air, even in the 'Subdivided Arena' experiment. Thus, a prey individual might not perceive a difference between the cue-less and cue-bearing half of the arena. In contrast, a strong difference will be perceivable for non-volatile cues. If the prey senses and reacts to both volatile and non-volatile cues, reactions will differ strongly between the two experimental setups, but the effects will be hard to predict. For future studies, this stresses the necessity to conduct both, and possibly additional, types of experiments in order to mimic the natural situation as close as possible.

Our study shows that antipredator behavior in wood crickets depends on the degree of threat posed by the respective predator, as has also been shown in avian and mammalian prey species (Caro 2005). Wood crickets showed two different behavioral reactions to predator cues – increase in mobility, in order to flee, and decrease in mobility, to remain undetected. They adjusted strength and the type of antipredator behavior to the predator species, its size and commonness. This coincides with Helfman's threat-sensitive avoidance hypothesis (1989) and Lima and Bednekoff's predation risk allocation hypothesis (1999), which predict that prey individuals should

adjust their behavior to the degree and type of predation threat they currently experience.

2.6. Acknowledgements

2.7. Appendix

Appendix A2.1: Cursorial spider species used in this study. Prosoma/Thorax widths, body lengths and weights are mean values (± standard deviation) of the respective spider and corresponding wood cricket species.

Spider family	Spider species	Spider code	Spider prosoma width [mm]	Spider body weight [mg]	Cricket thorax width [mm]	Cricket body weight [mg]
Agelenidae	*Agelena labyrinthica*	Al	2.19 (± 0.22)	42.14 (± 12.79)	1.93 (±0.20)	21.84 (± 4.99)
Amaurobiiade	*Amaurobius fenestralis*	Af	2.52 (± 0.45)	52.11 (± 26.68)	2.21 (±0.32)	40.54 (± 23.18)
Dysderidae	*Dysdera erythrina*	De	2.74 (± 0.30)	47.90 (± 19.55)	2.32 (±0.33)	51.56 (± 25.34)
Gnaphosidae	*Drassodes cupreus*	Dc	2.6 (± 0.98)	83.22 (± 61.96)	2.24 (±0.28)	44.98 (± 20.79)
Lycosidae	*Aulonia albimana*	Aa	1.36 (± 0.12)	10.93 (± 2.73)	1.90 (±0.14)	20.02 (± 4.71)
Lycosidae	*Alopecosa pulverulenta*	Ap	2.41 (± 0.27)	37.23 (± 8.02)	2.20 (±0.28)	35.35 (± 15.45)
Lycosidae	*Pardosa lugubris group*	Pl	2.27 (± 0.29)	23.77 (± 7.70)	2.35 (±0.33)	45.41 (± 23.17)
Oxyopidae	*Oxyopes ramosus*	Or	1.48 (± 0.19)	9.05 (± 4.21)	2.26 (±0.37)	42.24 (± 22.48)
Philodromidae	*Tibellus oblongus*	To	1.89 (± 0.42)	14.71 (± 11.85)	2.20 (±0.30)	38.06 (± 18.93)
Pisauridae	*Pisaura mirabilis*	Ad Pm	3.30 (± 0.20)	66.20 (± 14.05)	2.31 (±0.34)	44.20 (± 19.02)
		Im Pm	1.87 (± 0.28)	15.75 (± 5.06)	1.38 (± 0.15)	7.67 (± 2.56)
Tetragnathidae	*Pachygnatha degeeri*	Pd	1.15 (± 0.06)	5.47 (± 0.78)	1.97 (±0.18)	19.76 (± 5.14)
Theridiidae	*Enoplognatha ...*	Eo	0.97 (± 0.11)	6.68 (± 2.08)	1.94 (±0.15)	20.22 (± 4.13)
Thomisidae	*Xysticus cristatus*	Xc	2.31 (± 0.26)	24.90 (± 12.50)	2.38 (±0.40)	43.14 (± 21.21)
Zoridae	*Zora spinimana*	Zs	1.54 (± 0.37)	10.92 (± 7.14)	2.38 (±0.41)	50.51 (± 27.46)

3.

Behavioral response of a generalist predator to chemotactile cues of two taxonomically distinct prey species

Wood ants (*Formica polyctena*) feeding on mashed crickets and spiders.

© Hellena Binz

This chapter is based on an original research by

Binz H., Kraft E.F., Entling M.H. and Menzel F.

3.1. Abstract

Chemotactile cues left behind by animals in the ecosystem, can play a major role in shaping predator-prey interactions. Based on such cues, specialized predators can find their prey, while prey individuals can assess predation risk. However, little is known to date about the importance of chemotactile cues for generalist predators such as ants. Here, we investigated the response of a generalist predator, the wood ant *Formica polyctena*, to cues of two taxonomically distinct prey species: (i) nursery web spiders (*Pisaura mirabilis*), representing an intraguild prey, and (ii) wood crickets (*Nemobius sylvestris*). In analogy, we studied the response of crickets and spiders to ant cues. *Formica polyctena* workers changed movement patterns in response to cues of the two prey species, which can be interpreted as prey search behavior. The behavioral change did not differ between the two prey species, which coincided with similar aggression towards dead crickets or spiders, and equal consumption of dead crickets and spiders by the ants. However, although both crickets and spiders were strongly attacked by the ants, they showed no detectable antipredator behavior in response to ant cues. Our study shows that ants use chemotactile cues for prey detection and foraging. To our knowledge, the use of chemotactile cues for prey detection has not been shown in generalist predators yet, and may represent one of the causes for the high hunting efficiency of ants.

3.2. Introduction

Animals can use a variety of cues and signals of other individuals. Besides intended communication signals that directly transmit information between individuals (e.g. direct acoustic (Durant 2000), vibrational (Evans et al. 2009), visual (Hunter et al. 2007) and olfactory signals (McDougall & Milne 1968), there are also cues unintended left by different animals, which can influence other species' movement decisions. For example, many animals deposit different kinds of metabolic products, such as hair/feathers (Ekner & Tryjanowski 2008), silk (Rypstra & Buddle 2013), urine (Baudoin et al. 2013), feces (Agarwala et al. 2003) and footprints (Eltz 2006). Such 'deposits' can contain specific information about an individual's life history, e.g. taxon (Jędrzejewski et al. 1993) and size (Persons & Rypstra 2001; Binz et al. 2014b) or recent place of residence (Kats & Dill 1998). Other animals, given that they can detect and interpret those cues, can also use them to evaluate predation risk or to locate prey, and adjust their behavior accordingly (Dall et al. 2005): predators can search and detect their prey (Hughes et al. 2010) whereas prey can avoid locations that bear a high risk of predation (Persons et al. 2002). Moreover, dominant species can eavesdrop cues of subordinate species to kleptoparasitize their food sources (Creel et al. 2001; Nieh et al. 2004) or may approach them in order to maintain their territory boundaries against intruders (Binz et al. 2014a) . Competitively inferior species, in contrast, use cues of dominant species to avoid costly, aggressive encounters (Binz et al. 2014a). Here, the use of chemotactile cues can be a mechanism of coexistence *sensu* Chesson (Chesson 2000), enhancing local species richness. Hence, chemotactile cues can strongly affect a community's network of intra- and interspecific interactions and thus their ecosystem functions.

Accordingly, chemical oriented predators can drastically increase their foraging efficiency if they respond to chemotactile prey cues (Hughes et al. 2010). Various mammal predators indeed use their preys' intraspecific scent marks for prey search (Hughes et al. 2010). The same is true for specialist arthropod predators, such as ant-eating spiders (Cárdenas et al. 2012) and parasitoid Hymenoptera (Clavijo McCormick et al. 2012). These parasitoids are often specialized on one or few herbivorous insect species and seek herbivore-induced volatiles emitted by the prey's host plant. For a

generalist predator, however, it seems unlikely that it can learn or adapt to the odors of all potential prey species. Thus, it remains open whether generalist predators' use chemotactile cues of potential prey species in prey search in general and whether they differentiate between different types of prey.

Being ubiquitous and numerous, ants are among the most important generalist predators on arthropods in terrestrial ecosystems, and affect the functioning of most terrestrial ecosystems (Folgarait 1998; Domisch et al. 2009). Their ecological success is owed largely to a sophisticated chemical communication system, which enables a highly efficient social structure of ant colonies. Chemical communication in ants is used for nestmate recognition (Menzel et al. 2009; Van Zweden & D'Ettorre 2010), establishing dominance hierarchies within a nest (Liebig 2010), organizing task allocation (Greene & Gordon 2003) and recruiting nestmates to food sources (Morgan 2009) or colony defense (Witte et al. 2007). Besides, chemical communication occurs between ants and their mutualists (Menzel et al. 2008, 2013; Lang & Menzel 2011). The high demand for olfactory sensing and identifying chemotactile signals is reflected on a genetic level: ants have several hundred olfactory receptor genes (Zhou et al. 2012), which by far exceeds the number of olfactory receptor genes known in any other insect. Given the high olfactory abilities of ants, one should expect that ants also use chemotactile cues to locate prey. Surprisingly, however, little is known to date about how ants locate and trace their prey, and how important chemotactile cues are for this task (Schatz & Hossaert-McKey 2010; Pearce-Duvet & Feener 2010). So far, the use of visual (landmarks, Collett and Collett 2002) and, in part, chemical cues has been reported for orientation outside the nest, but not for the localization of prey (Horstmann 1982; Rosengren & Fortelius 1986).

As described above, chemotactile prey localization has been shown for specialist predators, but it should be more difficult for generalist predators due to the high number of different prey cues. One of such generalist predatory ants is *Formica polyctena*. It is territorial and dominates wide parts of coniferous and mixed forests throughout Middle and Northern Europe (Savolainen & Vepsäläinen 1989; Seifert 2007). Its diet comprises of almost every aboveground arthropod found in its territory (Folgarait 1998; Domisch et al. 2009).

Prey species, in turn, can use predator cues to minimize predation risk. Indirect chemotactile cues of predators elicit specific antipredator behavior in many insect and spider species (Persons & Rypstra 2001; Bell et al. 2006; Bucher et al. 2014a; Binz et al. 2014b). Such antipredator behaviors are usually manifested in a change in prey activity (Persons et al. 2001; Storm & Lima 2008), e.g. in the wood cricket *Nemobius sylvestris* (Gryllidae, Orthoptera) and the nursery spider *Pisaura mirabilis* (Pisauridae, Araneae). Both species respond to chemotactile predator cues (Bucher et al. 2014a). Since both occur syntopically with *F. polyctena* (Van der Aart & De Wit, 1971, authors own obs.) and serve as its potential prey (Dangles *et al.*, 2005, authors own obs.), they should strongly benefit from a reduced predation risk by responding to chemotactile cues of *F. polyctena.*

In the present study, we investigated reciprocal behavioral responses among the wood ant *Formica polyctena* and its potential prey: the wood cricket *Nemobius sylvestris* and the nursery web spider *Pisaura mirabilis*. While crickets and spiders should show antipredator behavior in response to ant cues, ants could use the cues of these two species to trace them as prey. We determined ant responses to cues of spiders and crickets, as well as spider and cricket responses to ant cues, by comparing activity patterns between areas with and without cues (Ivins & Smith 1983; Petranka et al. 1987; Barreto & Macdonald 1999; Séquin et al. 2003). In addition, we conducted aggression tests to examine the potential threat represented by *F. polyctena* workers for each prey species. To test whether responses of *F. polyctena* to prey cues match their prey preference, we ran a cafeteria test, simultaneously offering mashed crickets and spiders to workers of *F. polyctena* in the field.

We predicted *F. polyctena* workers to show searching behavior in the presence of prey cues (reduced speed/forward movement and thus increased residence time but still mobile on the spot (Weier & Feener 1995)). The magnitude of behavioral changes should depend on the ants' prey preference. In turn, we predicted *N. sylvestris* and *P. mirabilis* to show antipredator behavior, i.e. to respond to cues of *F. polyctena* by either escaping from high risk areas (e.g. reduce residence time, increased relative activity) or avoiding possible detection by the predator (e.g. increase residence time, decrease

relative activity). Here, the magnitudes of behavioral changes should match the aggression received by the ant.

3.3. Methods

Species studied

All arthropods were collected in two mixed forest stands and adjacent meadows, between October 2012 and April 2013. Individuals of the wood ant *Formica polyctena* (hereafter referred to as 'ants') were collected from three different mounds near Mainz (49°57'43" N / 8°10'51" E) and immature wood crickets (*Nemobius sylvestris*, 5th to 7th instar, hereafter referred to as 'crickets') were captured in the vicinity of Landau (49°11'45" N / 8°19'11" E). Both sites are located in Rhineland-Palatine, South Western Germany. Immature nursery web spiders (*Pisaura mirabilis,* 5th to 7th instar, hereafter referred to as 'spiders') were collected on both sites. As wood ants are protected by law in Germany (BNatSchG § 41, 42), we only collected worker groups (ca. 200 individuals/mound; permit: Gestattungsvertrag 6./8.3.2012, local nature conservation authority, Mainz-Bingen, Germany). Ants from the same mound were kept together in plastic nest-boxes (17.5 x 23.5 x 9.5 cm) with a periodically moistened plastered floor and some original nest substrate (soil or plant material). Walls were coated with Fluon® to prevent ants from escaping. Ants were fed with honey and small parts of dead house crickets *ad libitum*. Crickets were kept together in a plastic terrarium (30x20x20 cm) moistened with plaster of paris and fed with apple, carrots and cricket food *ad libitum*. Spiders were maintained singly in 40 ml glasses with moistened plaster of paris as ground in a climatic chamber (16 °C, 65% humidity and natural day/night rhythm) and were fed with 2-4 *Drosophila hydei* twice a week. Before each experiment we measured size (prosoma width in spiders, thorax width in insects) for each spider and cricket individual. Ant sizes were gained by averaging head widths of ten individuals per mound. At the end of the experiments, all arthropods were released in their original habitats.

Chemotactile cue experiments

In behavioral assays, we tested the response of these species to chemotactile cues from other species. Filter papers with cues of crickets or spiders ('cue donators'), respectively, were presented to ants ('reacting species') (N = 59 and 90). Vice versa, ant cues were presented to crickets and spiders as reacting species (N = 60 and 90) (see Binz *et al.*, 2014 for the behavioral response of crickets to spider cues). We used the experimental design of Persons and Rypstra (2001). The arenas were covered with two semi-circles of filter paper, one with cues and one without. The arenas themselves consisted of a 15 cm high reusable plastic tube with a diameter of 10 cm, coated with Fluon® to prevent animals from escaping. A Petri dish served as exchangeable arena floor. Chemotactile cues were obtained by keeping either one spider, three crickets or three ant individuals from the same colony separately on two moistened filter paper halves during 48 hours directly prior to the experiment. We used three crickets or ants instead of one since they will rarely be encountered alone in nature. The control filter paper halves were treated the same way, but without any animal contact. After removing the animals, filter paper halves were placed on new Petri dishes at the bottom of the arenas and combined with untreated halves. Thus, always two semi-circles of filter paper, one containing chemotactile cues and one serving as control, covered the ground. One individual of the tested species was then placed into the center of one arena and the arena was closed with a glass plate. After two minutes of acclimation, six arenas and thus six independent animals were simultaneously filmed for 15 minutes with a video camera (SONY HDR-CX 550 VE) mounted on a tripod.

We used the tracking software EthoVision XT8 (Noldus Information Technology, Wageningen, The Netherlands) to automatically track the activity and location of the animals on the filter papers during the experiment, with 5 frames per second. We analyzed six activity parameters: residence time, time spent immobile, mobile and highly mobile, forward walking and mean speed on each paper half. The residence time on each paper half was recorded in seconds (0 – 900 sec). If the total area covered by the animal changed less than 30% pixels per frame, it was recorded as immobile. 'Time spent mobile' was defined as the time when 30 to 70 % of the pixel (which represented the animal) changed per frame. 'Time spent highly mobile' was defined as the time when pixel change exceeded 70 %. Such mobility measures include non-directed

movements such as turning. Forward movement ('walking forward') was recorded if the center of the animal moved more than half the length of the animal per second (ants: 0.40 cm/s; crickets: 0.45 cm/s; spiders: 0.38 - 0.47 cm/s, depending on individual size). For statistical analyses, we used relative residence times and adjusted activities to the residence time on the respective filter paper halves. Mean speed was directly calculated by the software by dividing distance walked by residence time (cm/s). We thus obtained a total sample size of N = 598 (cue donator - reacting species: 'cricket – ant': N = 120; 'ant – cricket': N = 118; 'spider – ant' and 'ant – spider': N = 180, respectively).

We then analyzed the difference of each activity parameter between control and cue-bearing filter paper for each species combination separately using linear mixed effect models (LME). Moreover, also using linear mixed models, we tested if the behavioral changes of the ants differed between the different cues of prey species. Each model included three random factors: 'arena_ID', 'cue_ID' and 'reacting_ID'. 'Arena_ID' was assigned to the two filter paper halves of each experimental arena ('cricket – ant' = 60 arenas and 'ant – cricket' = 59 arenas, 'spider – ant' and 'ant – spider' = 90 arenas, respectively). The random factor 'cue_ID' considered the two filter paper halves that had been covered with cues of the same animal(s) ('cricket – ant' and 'ant – cricket' = 30 groups of two halves, respectively, 'spider – ant' and 'ant – spider' = 45 groups, respectively). Lastly, the 'reacting_ID' contained the affiliation of the tested animals towards a population in crickets ('ant – cricket' = 1 population), a colony in ants ('cricket – ant': 1 colony, 'spider – ant': 3 colonies) or individuals in spiders ('ant – spider': 60 individuals).

Aggression tests

We compared relative aggression of ant workers towards live as well as dead cricket and spider individuals. Testing *F. polyctena* aggression towards dead *N. sylvestris* and *P. mirabilis* revealed the aggression level towards the prey's chemical profiles only, without the stimuli resulting from prey movement and defense. During three minutes, we observed all interactions performed by three ant workers towards one of the four prey items: 'live cricket' (n = 20), 'live spider' (n = 20), 'dead cricket' (n = 18) or 'dead spider' (n = 18). Workers were freshly collected from 20 different domes (< 50 workers

per dome). Aggression tests were conducted during March 2013 using the same arenas as in the video setup. We scored antennation (a) as nonaggressive, and five other behaviors as aggressive interactions: mandible spreading (m), biting (b), holding (h), dragging (d), and gaster lifting (g). For each assay, an aggression index was calculated as the ratio of aggressive interactions to all interactions, where weakly aggressive interactions (mandible spreading and gaster lifting), that did not involve physical contact, were scored half, viz.: $\frac{\frac{m}{2}+b+h+d+\frac{g}{2}}{a+m+b+h+d+g}$ (modified after Pamminger *et al.*, 2011).

Using linear mixed models (LME) we tested for differences in ant aggression between the four prey items. Additionally, after square-root-transformation to reduce heterogeneity of variances, mean differences in total number of all interactions of ants towards the four prey items was tested using linear mixed models (LME). As we tested each ant colony once against all four prey items, we added 'colony_ID' (= 20 colonies) as random factor.

Food preference test

We tested for a possible prey preference of the ants by offering mashed crickets or spiders to nine independent ant mounds. We put two separate Petri dishes (Ø 3.5 cm), one containing mashed crickets, the second mashed spiders (167 ± 18 mg SD), next to one of the main routes of each mound. Ants were then allowed to freely choose and feed on both types of prey during the next 10 minutes. An additional empty Petri dish served as weight control for microclimatic influences on Petri dish weight. We measured weights of the three petri-dishes before the start of the experiment and again 10 minutes later, directly after the end of the experiment. We then calculated the deviation in prey weight, including the weight difference of the control Petri dish (n = 9 * 2).

Every minute after the start of the experiment we additionally took photos of each Petri dish containing mashed prey and counted those ant individuals that fed on the mashed prey for each species and time, respectively. The recruitment rate was then calculated by the mean change of ant numbers at each prey during the ten minutes (n = 9 * 2, with 10 measures per replicate).

Using linear mixed models (LME) with 'colony_ID' as random factor (= 9 colonies), we tested which prey species was consumed more and to which prey species the ants

recruited more workers. All statistical analyses were based on normally distributed data and calculated in R 3.0.2 (R Development Core Team 2013) using the packages MASS (Venables & Ripley 2002), lme4 (Bates et al. 2012), nlme (Pinheiro et al. 2012) and sciplot (Morales & R Development Core Team 2012).

3.4. Results

Chemotactile cue experiments

Ants similarly reacted towards spider and cricket cues. They spent more time on the filter paper half covered with cues of either crickets (LME: $t_{1,59}$ = 4.70, p < 0.001) or spiders (LME: $t_{1,89}$ = 3.65, p = 0.001, Fig. 3.1 a) than on the untreated filter paper half. Moreover, they increased the relative time spent immobile (LME: crickets: $t_{1,59}$ = 2.26, p = 0.028; spiders: $t_{1,59}$ = 2.53, p = 0.013, Fig. 3.1 b) and walked relatively less (LME: crickets: $t_{1,59}$ = - 2.34, p = 0.023; spiders: $t_{1,89}$ = - 2.98, p = 0.004, Fig. 3.1 c) when detecting chemotactile cues of prey. In addition, the chemotactile cues of prey induced a reduced mean speed (LME: crickets: $t_{1,59}$ = - 2.87, p = 0.006; spiders: $t_{1,58}$ = - 3.26, p = 0.002, Fig. 3.1 e) and ant workers also spent relative less time highly mobile (LME: crickets: $t_{1,59}$ = - 3.07, p = 0.003; spiders: $t_{1,89}$ = - 3.38, p = 0.001, Fig. 3.1 f). However, they did not change their relative time mobile between filter paper halves (LME: crickets: $t_{1,59}$ = - 0.61, p = 0.54, Fig. 3.1 d). The ants' activity measures did not differ between cues of spiders and crickets (LME: all six p > 0.29). In contrast to the ants, neither crickets nor spiders, showed any changes in activity and/or residence time in response to chemotactile cues of the ants (all p > 0.18, Fig. 3.1 a-f).
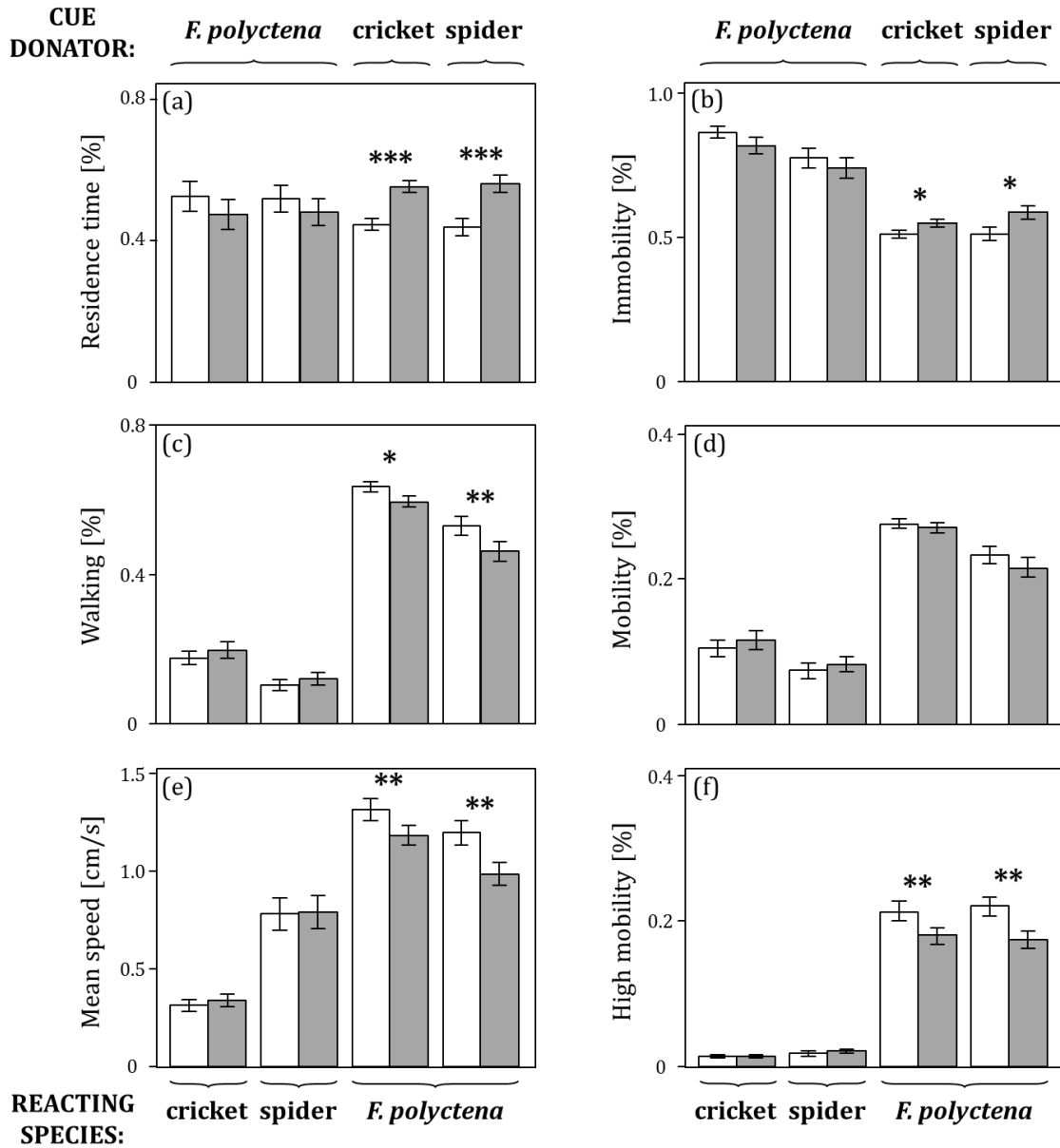
Figure 3.1: Mean activities (± SD) on filter paper halves covered with chemotactile cues (grey bars) and on clean filter paper halves (white bars): relative residence time (a), mean speed (e) as well as time spent immobile (b), walking (c), mobile (d), and highly mobile (f). The metrics (b), (c), (d) and (f) are each relative to the total residence time on the respective filter paper halves. Species names at the top indicate the origin of the olfactory cues covering one filter paper half. Species names at the bottom indicate the tested species. 'cricket' = *Nemobius sylvestris*; 'spider' = *Pisaura mirabilis*. *N* = 598. Significance levels are based on linear models: ***P < 0.001, **P < 0.01, *P < 0.05

Aggression tests

Ant workers were more aggressive towards live spiders than towards live crickets (LME: $t_{1,53}$ = 3.94, p = 0.0002, Fig. 3.2 a). Generally, aggression towards dead prey individuals was lower than for live prey (LME: $t_{1,55}$ = - 6.25, p < 0.0001) and did not differ between crickets and spiders (LME: $t_{1,53}$ = 0.23, p = 0.816). Living spiders elicited by far more interactions with ants than live crickets or dead prey items (LME: all three $t_{1,53}$ > 6.23, all three p < 0.001, Fig. 3.2 b), with which the ants interacted equally (LME: all three: $t_{1,53}$ < 1.41, all three p > 0.16).
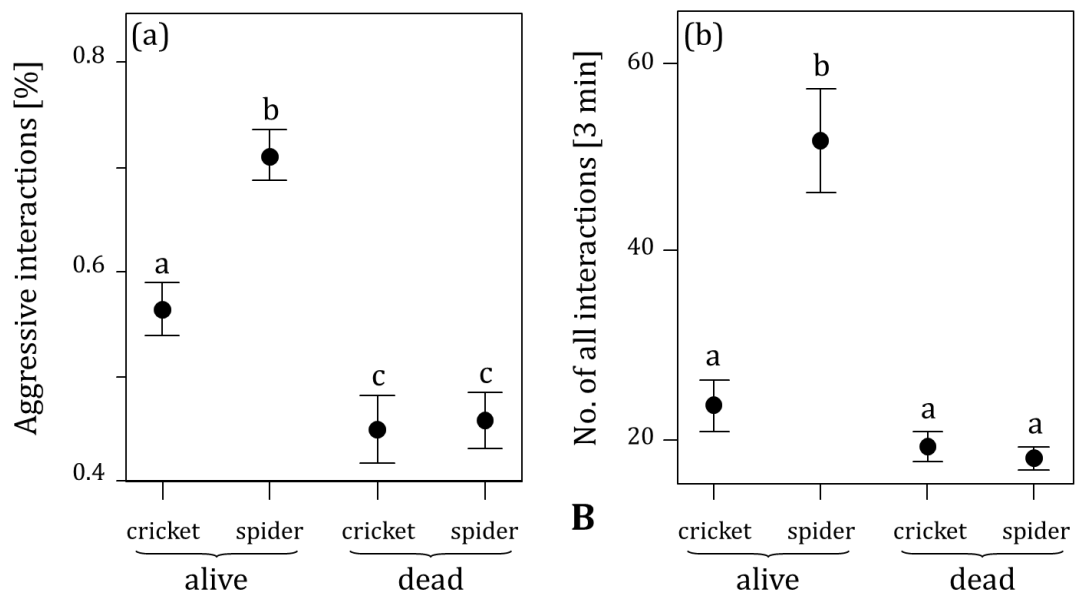

Figure 3.2: Mean (± SD) relative aggression (a) and total number of interactions (b) towards live or dead wood cricket (*Nemobius sylvestris*) and spider (*Pisaura mirabilis*) individuals by three *Formica polyctena* during three minutes. Different letters indicate significant differences at ⍺ < 0.05, based on linear mixed models (a) and generalized mixed models (b).

Food preference test

Ant workers consumed similar amounts of mashed cricket and spiders (LME: $t_{1,8}$ = - 0.55, p = 0.60, Fig. 3.3 a) and recruited equally to both prey species (LME: $t_{1,170}$ = - 0.24, p = 0.81; Fig. 3.3 b).
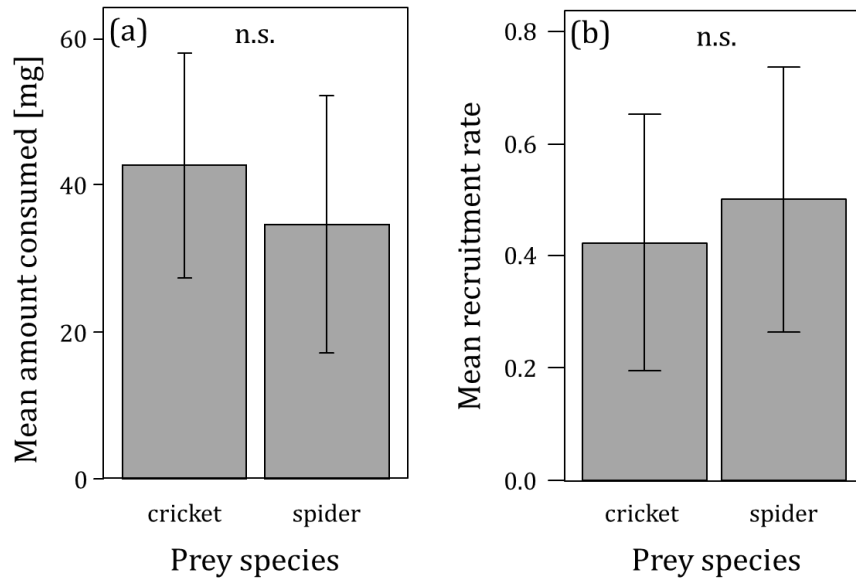
Figure 3.3: Mean amount of mashed wood crickets (*Nemobius sylvestris*) or spiders (*Pisaura mirabilis*) consumed (± SD) by *Formica polyctena* (a) from nine different mounds during ten minutes and mean number of *F. polyctena* workers recruited to the respective prey species (b). The difference between prey species is not significant.

3.5. Discussion

Our study revealed that workers of the wood ant *Formica polyctena* respond to chemotactile cues of potential prey species and presumably use them to increase their search efficiency. The strength of their responses did not differ between *Pisaura mirabilis* (nursery web spider, an intraguild prey), and *Nemobius sylvestris* (wood cricket), although they are taxonomically distinct and leave chemically different cues (authors unpublished data). This coincides with the similar aggression levels towards dead crickets or spiders, and similar recruitment to, and consumption of, carrion of the two species. In contrast, neither *N. sylvestris* nor *P. mirabilis* responded towards chemotactile cues of their predator *F. polyctena*.

Formica polyctena reacts to chemotactile prey cues
Workers of the wood ant *F. polyctena* showed strong and indistinguishable responses towards cues of the two prey species *N. sylvestris* and *P. mirabilis*, indicating that can detect prey cues. The ants stayed longer in cue zones and reduced their speed and forward movement, but did not change their mobility compared to the cue-free filter

paper half. This suggests that *F. polyctena* performed prey-search behavior (Weier & Feener 1995).

That ants use chemotactile cues to hunt for prey has only been shown rarely up to now (Schatz et al. 2003). As described above, it is well known that ants use chemotactile cues for a wide variety of functions, including recruitment to food sources, but their use for prey search has been little studied up to now. Chemotactile cues are used by several insect herbivores searching for host plants, and by certain parasitoids (Clavijo McCormick et al. 2012). These herbivores and parasitoids are usually specialized on few host species, and therefore only need to react to a narrow range of chemical cues. The same is true for specialist predators (Cárdenas et al. 2012). In contrast, *F. polyctena* is a generalist predator (Hawes et al. 2002; Seifert 2007). Thus, it should be more difficult to learn or adapt to chemotactile cues of all potential prey species in its foraging range. Nevertheless, *F. polyctena* reacted to prey cues, but did not discriminate between cues of different prey. Although *N. sylvestris* and *P. mirabilis* carry highly different cues (i.e. different cuticular hydrocarbons; FM & HB unpublished data), *F. polyctena* did not show any behavioral difference, neither in reactions towards cues nor in food preference, recruitment rate or aggression towards dead individuals. It seems likely that *F. polyctena* can detect chemical differences in cues of different prey species. Furthermore, spider cues also include a physical component (silk, Rypstra and Buddle 2013). Nevertheless, our results suggest that they show a standardized prey search behavior as a response to all non-nestmate chemotactile cues while foraging.

Lack of ant avoidance in crickets and spiders

Ants attacked both wood crickets and nursery web spiders, and fed on dead, mashed individuals of both species. However, neither crickets nor spiders showed detectable antipredator behavior in response to ant cues. It has been shown before that the two species react to chemotactile cues: wood crickets showed antipredator behavior towards cues of spiders (Binz et al. 2014b), and spiders reacted towards cues of conspecifics (i.e. potential predators, Bucher et al. 2014). The lack of reaction towards ant cues might be due to ubiquity of ant cues. *Formica polyctena* is a highly territorial ant, which constantly marks and defends their territory and the including food sources

(Hölldobler & Wilson 1977, 1990). When their colonies are dense, wide parts of the forest floor should be covered with ant cues. Thus, cue presence may not be accurate enough to predict the current presence of ants in the direct vicinity. Prey species might therefore rather rely on mechanical, visual or vibrational predator cues. Using their cerci, crickets can perceive approaching predators via air movements (Dupuy et al. 2011), and may therefore be able to escape ant attacks by jumping away. Indeed, individual ants often had trouble to bite or grab the crickets before they jumped out of reach in the aggression assays. Spiders, on the other hand, are hard to detect by the crickets as they approach either at high speed (~30 cm/s) to catch them on the hop, or at low speed (<6 cm/s) to avoid airborne vibrations (Dangles et al. 2006). This may explain why crickets show antipredator behavior against spiders (Binz et al. 2014a) but not against ants.

Spiders, on the other hand can sense approaching predators from substrate vibrations (Hergenröder & Barth 1983) or by vision (Clemente et al. 2010). The nursery web spider normally evades predation by climbing up plants and run over the vegetation, leaving the slower predator behind (HB, personal observation, Roberts, 1996). However, this was not possible in the arenas used for the aggression assays, thus making it easy for the ants to bite and grab the spider, which accounts for the high aggression between ants and living spiders. *Pisaura mirabilis* possesses large quantities of hydrocarbon on its cuticle (HB & FM, unpublished data), which makes it likely that ants can perceive them (Uhl 2013). At the same time, *F. polyctena* leaves relatively large hydrocarbon quantities as chemical footprints (FM unpublished data). In view of optimal risk avoidance (Ydenberg & Dill 1986), the attack rate by ants and ant avoidance by other spider species (Mestre et al. 2014), the lack of response to ant cues by spiders is thus hard to explain.

3.6. Conclusion

Our study shows that workers of the wood ant *F. polyctena* respond to cues left by potential prey species. It seems likely that incorporating these cues into foraging decisions enables *F. polyctena* to increase their foraging efficiency. The ability of ants to detect and respond to these cues may stem from their complex olfactory communication, which is tightly linked to their eusocial organisation. The use of chemotactile cues in ant predation is a little-studied field up to now, but opens up intriguing research questions. Being a generalist predator requires that ants discriminate a variety of prey cues as different as those of spiders and crickets from irrelevant cues, e.g. of plants, which often also carry hydrocarbons (Eigenbrode & Espelie 1995). Future studies should aim to elucidate the specificity of ant responses to chemotactile cues, the ability of ants to differentiate between chemotactile cues of different prey species, and whether such prey cues are learned or innate.

3.7. Acknowledgements

4.

The chemistry of competition:

Exploitation of heterospecific cues

depends on the dominance rank in the

community
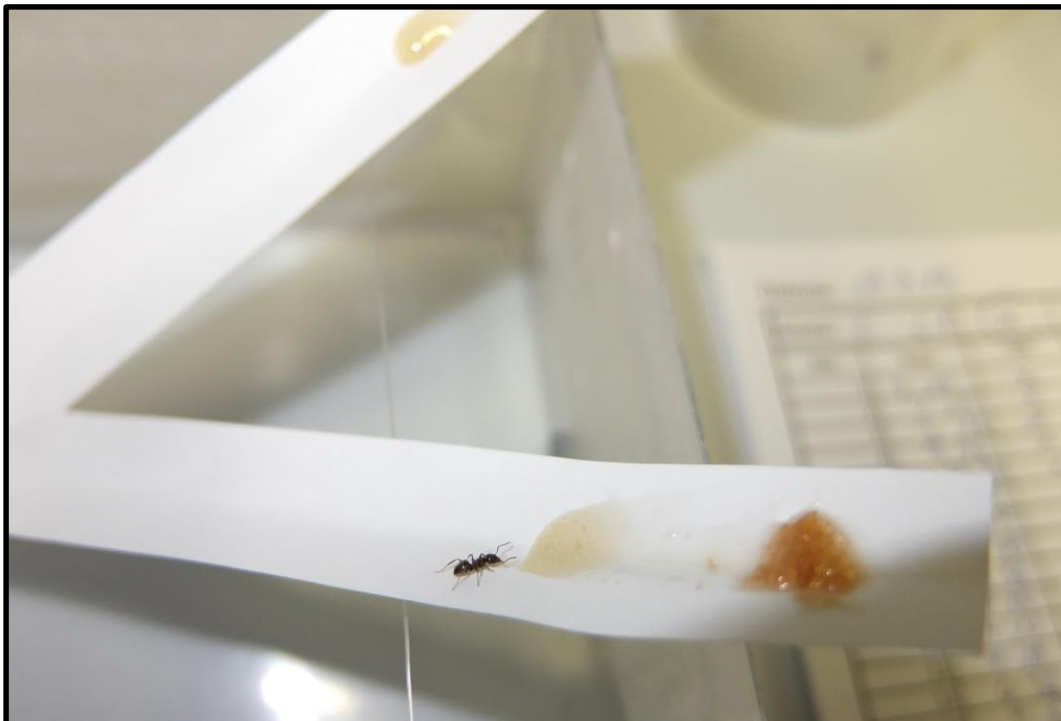
A worker of the black garden ant *Lasius niger* participating in the Y-maze
assay.

© Hellen Binz

This chapter is based on an original research article published in *Animal Behaviour* in August 2014:

4.1. Abstract

Interspecific competition is an important ecological mechanism shaping the traits of the interacting species and structuring their communities. Less competitive species benefit from evading direct encounters with aggressive dominants, whereas dominant species could use cues left by subordinates to steal their resources or to chase them off. Here, we studied competitive interactions among five common and syntopic ant species in Central Europe (*Formica polyctena*, *Formica rufibarbis*, *Lasius niger*, *Myrmica rubra* and *Tetramorium caespitum)* and investigated their ability to react to heterospecific chemical cues. Using aggression assays, we established a clear dominance hierarchy of these species, with *L. niger* and *F. polyctena* as the most dominant species. Using Y-mazes, we then tested whether ants avoid or prefer areas with cues of either dominant or subordinate species. These cues included trail pheromones, cuticular hydrocarbons and chemical footprints. Ants of all species ignored heterospecific trail pheromone extracts, but two of the three subordinate species avoided cuticular hydrocarbons of the dominant species. In contrast, dominants either ignored or were attracted to cuticular hydrocarbon extracts of subordinates. The avoidance behavior of the subordinates might be quantity-dependent, as footprints of the dominant species *L. niger* attracted two subordinates. The lowest ranking species *M. rubra* was unresponsive to cues of heterospecifics but avoided following the traces of their own colony members. Our study shows that ants exploit heterospecific cues either to avoid or to seek competitors and that their reaction depends on their dominance rank in the local community.

4.2. Introduction

Interspecific competition is a widespread phenomenon that influences population dynamics and community structure (Gibb & Johansson 2011; Dhondt 2012), thereby exerting selection pressure on many species. This in turn results in the evolution of differentiated niches characterized, for example, by different habitat or diet requirements (Dayan & Simberloff 2005; Stuart & Losos 2013). Nevertheless, niches of co-occurring species often partially overlap, leading to competition, which can be indirect or direct. In the former case, a species exploits a shared resource without directly encountering the other species. However, heterospecific competitors often directly face each other in aggressive encounters (Bicca-Marques & Garber 2003; Vonshak et al. 2011). The species with the best fighting abilities will dominate the respective resources and aggressively displace subordinate species (Rowland 1983). To counteract the fitness costs of being outcompeted, subordinates need alternative strategies to be able to co-occur with dominants. Besides niche differentiation, subordinates often exploit visual (Hunter et al. 2007), acoustic (Durant 2000) or vibrational (Evans et al. 2009) cues to detect and avoid dominant competitors. However, indirect cues, such as chemical traces unintentionally left behind, have the advantage that they are more persistent than direct cues and can therefore indicate the prior presence of competitors (Kats & Dill 1998). Such olfactory cues play an important role in antipredator behavior of prey organisms (Caro 2005; Schmitz 2008b; Binz et al. 2014b). However, their role in interspecific competition has been addressed by few studies to date, and they involved only two competitors at a time (Polo-Cavia et al. 2009; Baudoin et al. 2013). Like olfactory hunting predators that use chemical cues to detect and locate their prey (Hughes et al. 2010), dominant species could also seek subordinates via olfactory cues either to prey upon them (Schatz & Hossaert-McKey 2010; Carthey et al. 2011), to steal their food (kleptoparasitism: Creel, Sprong, & Creel, 2001; Nieh et al., 2004) or to displace them from their territory. Olfactory cues released by subordinates and/or dominant competitors could therefore influence interspecific competition and consequently, as in predator–prey interactions, affect community dynamics.

In ants, interspecific competition is particularly pronounced (Parr & Gibb 2012), as 'the worst enemy of an ant is another ant' (Forel 1874, p. 452). Ant communities are usually structured into dominance hierarchies (Fellers 1987; Parr & Gibb 2010), with dominant species aggressively displacing subordinates from resources in direct behavioral interactions (Cerdà et al. 2013). Therefore, information on the taxonomic affiliation of an opponent can be of major importance for an ant species. However, interspecific competition in social species and especially in ants may depend not only on the respective species present at a site, but also on their relative abundance (Helfman 1989). Owing to their diverse chemical communication system, ants are ideal to investigate the role of chemical cues in interspecific competition (Hölldobler & Wilson 1990). These social insects use cuticular hydrocarbons to distinguish nestmates from non-nestmates, but they can also recognize other species based on their chemical profiles (Drescher et al. 2011; Lang & Menzel 2011), which are species-, colony- and often even caste-specific (Howard et al. 1982; Bagnères et al. 1998). By walking on receptive surfaces, insects unintentionally leave 'chemical footprints' behind, which are congruent to the individual's cuticular profile (Akino & Yamaoka 2005; Devigne & De Biseau 2012) and which can be detected by others (Eltz 2006; Cárdenas et al. 2012). These chemical footprints should therefore contain reliable information on the recent presence and workforce of other species. For example, weak cues such as footprints indicate the presence of a few individuals, whereas more intense cues such as the extracts of cuticular hydrocarbons might mimic the presence of multiple individuals. Hence, subordinate species might display different responses to cues of varying intensity. Many ants also deposit pheromone trails to recruit nestmates to food sources, and these pheromones are usually species- or genus-specific (Czaczkes et al. 2013). Ant workers of several species have been shown to detect heterospecific trail pheromones and to follow them to food sources (Morgan 2009; Menzel et al. 2010b). If subordinate species were similarly able to read the trail pheromones of dominant species, they could use these chemical cues to circumvent competitive encounters.

Here, we studied the interactions among five common and sympatric ant species. We examined whether they react to chemical cues of competing species and whether their response is associated with their dominance rank. First, we determined the

dominance hierarchy among the syntopic ant species. Then, Y-maze assays were used to test for the ants' responses to cues by dominants or subordinates. Cuticular hydrocarbon extracts, owing to their similarity to footprints, provide strong evidence for the activity of other species. We thus predicted that subordinate species should avoid cues of dominant species to prevent competitive encounters. Dominant competitors, in contrast, were expected either to ignore subordinates' cues, as they do not pose a threat, or even to be attracted by them in order to exploit the subordinate's food resources, to defend their territory or to prey on them. Our expectations about the response of subordinates to trail pheromones were similar to those for their response to cuticular hydrocarbons, albeit these cues might be less species-specific. Finally, we studied whether the lower quantities of dominants' footprints, in contrast to cuticular hydrocarbon extracts, induce a less pronounced response in subordinates.

4.3. Methods

Study organisms

We investigated interactions among five common and co-occurring species: *Formica polyctena* (Formicinae), *Formica rufibarbis* (Formicinae), *Lasius niger* (Formicinae), *Myrmica rubra* (Myrmicinae) and *Tetramorium caespitum* (Myrmicinae). All five species can have large colonies with between 1000 and 6 million workers, are widespread throughout Europe with overlapping habitats and food sources (Seifert 2007) and use trail pheromones for mass food recruiting (Cammaerts-Tricot et al. 1977; Horstmann et al. 1982; Attygalle & Morgan 1985; Beckers et al. 1993; Cárdenas et al. 2012). On a meadow near Mainz, Germany, we collected 10 colonies each of *F. rufibarbis*, *L. niger* and *T. caespitum*. In addition, 10 colonies each of *F. polyctena* and *M. rubra* were collected in a mixed forest 1.5 km from the first site. As *F. polyctena* is protected by law in Germany (BNatSchG § 41, 42), we could only obtain a permit to collect worker groups (300 ± 80 individuals/mound; Gestattungsvertrag 6./8.3.2012, local nature conservation authority, Mainz-Bingen, Germany). Workers from the mound's surface, which are known to be the older and more experienced workers (so called 'observer workers'; Savolainen, Vepsäläinen, & Wuorenrinne, 1989) were

collected a day before the experiments. We dug up entire colonies of the other study species using spades, and transferred them to the laboratory. In some cases, we were only able to obtain the workers but not their queen. Queens were present in four of the 10 *T. caespitum* nests, four of the 10 *F. rufibarbis* nests and none of the 10 *L. niger* nests. For the strongly polygynous *M. rubra,* queen numbers ranged from one to 27 (median 5.5). As brood presence is known to influence activity and stimulate foraging (Vowles 1952; Ravary et al. 2006), all colonies (except of the *F. polyctena* worker groups) contained brood. Colony sizes of *F. polyctena* were calculated using a ground layer formula (provided by B. Seifert, Senckenberg Museum of Natural History Görlitz, Germany):

F. polyctena colony size = (0.414 x ground area in m^2 + 0.119) x 1 000 000   (1)

For the other species, we counted all workers in the colonies after the end of the experiments. Colony sizes differed strongly between species and colonies (*F. polyctena*: 2 602 586 ± 421 782 SE; *F. rufibarbis*: 1202 ± 240; *L. niger*: 1157 ± 169; *M. rubra*: 276 ± 42; *T. caespitum*: 2969 ± 555). Ant colonies were transferred to and kept in plastic nestboxes (17.5 x 23.5 cm and 9.5 cm high) with a periodically moistened plaster floor and original nest substrate (soil or plant material) covering half of the floor. Walls were coated with Fluon to prevent ants from escaping. At the side of each nestbox, a plastic tube (10 x 1 cm) was fixed at ground level which served as a connection to other arenas. All ants that participated in the experiments had deliberately left their nest and walked through the plastic tube. We therefore assumed that all ants we tested were scouting foragers. Ants were kept on a restricted diet (three drops of honey per day) to stimulate scouting activity.

Interspecific aggression assays

Aggression between species was measured by confronting the colonies of the different species with colonies of the other species. Each of the five species was confronted with all the other species in a random order, resulting in a total of 10 species combinations. For each species, every colony was confronted once with one of the other species, resulting in 10 replicates per species combination. For such a confrontation, nestboxes of two heterospecific colonies were connected to the same neutral 'arena' (11.5 x 17.5

cm and 7 cm high plastic box with Fluon-coated walls). The connections consisted of plastic tubes (10 x 1 cm), which were located at the opposite short sides of the arenas. We placed a bait (four drops of honey mixed with ca. 0.1 g of tuna) in the centre of the arena and individuals of both species were then allowed to freely enter the arena through the connection tube and feed on the bait. We recorded the first 30 interactions and classified them as aggressive (attack with biting), threatening (mandible opening), defending (bite back after being attacked), neutral (antennate) and submissive (jerk back). Aggression assays were conducted in July 2013.

We focused our analyses on aggressive behaviors (i.e. aggression = mean number of attacks) as aggression is a manifestation of dominance (Cerdà et al. 1997; Santini et al. 2007). First, using linear mixed-effects models (LME) with Gaussian error distribution (R package nlme; Pinheiro et al., 2012), we tested for an influence of colony size, queen presence and number on aggression. Because each colony was tested four times in total (against one colony of each of the other four species), 'colony ID' was added as a random factor. As neither colony size (LME: $F_{1,48}$ = 1.20, P = 0.28), queen presence (LME: $F_{1,28}$ = 1.47, P = 0.24) nor queen number (*M. rubra* only: LME: $F_{1,8}$ = 0.25, P = 0.63) affected aggression, these factors were excluded from further analyses.

Using a similar LME with Gaussian error distribution, we then tested for differences in the aggression that each species inflicted on and received from all heterospecifics in total and between heterospecifics. There was no effect of queen presence (LME: $F_{1,28}$ = 1.47, P = 0.24) or queen number (*M. rubra* only: LME: $F_{1,8}$ = 0.25, P = 0.63) on aggression. Analysis based on aggressive and weakly aggressive interactions (threatening and defending; each scored only half according to their lower intensities sensu Pamminger et al. 2011) yielded similar results. We decided to employ the most commonly used term 'subordinate' for less competitive species with a lower behavioral rank in the community (Bicca-Marques & Garber 2003; Goodale et al. 2010; Dhondt 2012). However, note that in some of the ant literature, the term subordinate is used exclusively for species that are never attacked (e.g. Vepsäläinen & Pisarski 1982; Savolainen et al. 1989; Cerdà et al. 2013).

Extract assays with cuticular hydrocarbon or trail pheromone extracts

Using Y-maze assays, we tested whether ants chose a clean control path over a path on which cuticular hydrocarbon or trail pheromone extracts of different species were applied. In particular, we separately tested the choices of the two most dominant species towards extracts of subordinates that were significantly less aggressive and vice versa. We thus had two groups of dominants versus subordinates: (1) *L. niger* versus *T. caespitum*, *F. rufibarbis* and *M. rubra* and (2) *F. polyctena* versus *F. rufibarbis* and *M. rubra*. In the first assays, using cuticular hydrocarbon extracts, we tested the choices of subordinates and dominants against each other. In the second assays, using trail pheromone extracts, we only tested the choices of the subordinate species. We conducted 10 assays (one per colony) for each extract type and species. The cuticular hydrocarbon extracts were made by immersing five freeze-killed ants from the same colony in approximately 4 ml of hexane for 10 min. After removing the ants, we evaporated the extracts under a flow of nitrogen and then added 25 µl of hexane, resulting in a concentration of extracts of 5 ants/25 µl. Trail pheromones in formicine ants are produced in the hindgut (Morgan 2009). We therefore obtained the trail pheromone extract by dissecting hindguts of five freeze-killed individuals from the same colony, and immersing them in 25 µl of hexane. This method is standard practice to obtain standardized quantities of trail pheromones and test their biological activity (Attygalle & Morgan 1985; Morgan 2009; Menzel et al. 2010b).

All Y-maze assays were conducted in neutral arenas (plastic boxes: 11.5 x 17.5 cm and 7 cm high, walls coated with Fluon) that were connected to the nestboxes with a plastic tube (10 x 1 cm). As the ants had to leave their nestbox deliberately through the plastic tube to enter the neutral arena, we presumed them to be scouting foragers. Inside the neutral arenas, we put a Y-shaped paper sheet (bottom 9 cm and arms each 12 cm long) which was held up by a nylon cord. The nylon cord was fixed over the rim of the neutral arenas, 2 cm from the opposite site of the arena entrance so that ants could only enter the maze at the bottom. On each end of the two arms we put a bait (one drop of honey mixed with ca. 0.05 g of tuna) to entice ants into entering the maze. For each olfactory cue assay, we applied an extract onto one arm of a fresh Y-shaped paper sheet (bottom 9 cm and arms each 10 cm long). We coated the first 2 cm of one arm of the Y-maze with 25 µl of extract (extract side), and the first 2 cm of the other arm with

the 25 µl of hexane (control side). After the coating was applied, 15 scouting foragers were allowed to walk on to the sheet one at a time and we scored an ant's decision (left or right side) when it chose one of the two baits. As ants start laying trail pheromones after discovering food (Beckers et al. 1992), we immediately removed them from the Y-maze after they had made their choice, to prevent them from laying trail pheromones, and put them in a separate box until the end of the assay. Cuticular and trail pheromone extract assays were conducted during August and September 2013.

For each extract assay, we subtracted the number of ants choosing the extract side of the Y-maze from the number of ants choosing the control side, thus obtaining 10 values per species and group (one value for each assay). Positive values indicate avoidance and negative values attraction towards heterospecific olfactory cues. We first tested the influence of colony size, queen presence or number (in *M. rubra* only) and side preferences on the ants' choice using linear models for each group. However, none of these parameters affected the ants' behavior (colony size: all $P > 0.12$; queen presence: all $P > 0.10$; queen number in *M. rubra*: all $P > 0.28$; side preference: all $P > 0.11$). We then tested whether the choice of a branch was influenced by heterospecific extracts by using linear models (LM) with Gaussian error distribution and an intercept of zero (hence, no loss of degrees of freedom) for each extract type and group separately.

Control assays with footprints of nestmates

To assess the effect of potential footprint following of nestmates on the Y-mazes, we conducted one assay with untreated Y-mazes for each colony ($N = 5 \times 10 = 50$). As in the previous Y-maze assays, we allowed 15 scouting foragers to walk consecutively on to the sheet and we scored an ant's decision (follow previous ant or 'not', $N = 14$/colony as the first ants could not be assigned to a group) when it settled at one of the two baits. We then subtracted the number of ants following the previous ant from the ones 'not' following the previous ant for each colony, resulting in one value per assay and 10 values per species. Using linear models with an intercept of zero, we determined whether the choice of individuals on the same sheet was independent of each other or not. If individual choices were not independent of each other, we controlled the results

of the extract assays for a possible conspecific footprint effect using LMEs with Gaussian error distribution. These footprint control assays were conducted in August 2013.

Assays with footprints of dominant workers

Also using Y-mazes, we tested whether lower quantities of dominants' footprints, in contrast to cuticular hydrocarbon extracts, induce a less pronounced response in subordinates. We therefore tested the choice of footprints of *L. niger* by the two subordinate species that showed a response towards its cuticular hydrocarbon extracts (i.e. *F. rufibarbis* and *T. caespitum*). As *M. rubra* did not show a response to cuticular hydrocarbon extracts of *L. niger* it was not tested here. Single *Lasius japonicus* workers have been shown to release enough footprint hydrocarbons for identification in 10 min (Akino & Yamaoka 2005). Therefore, we obtained footprints by allowing 20 *L. niger* workers to walk from the tip of one arm of each Y-maze towards the fork. They were immediately removed when reaching the fork to prevent them from laying trail pheromones (Beckers et al. 1992). To carry out the choice assays, we carefully transferred 15 workers per colony (only those located outside the nest substrate and thus scouting foragers) into a small cup (diameter 8 cm) with Fluon-coated walls. After a few minutes to allow the ants to settle, we placed a Y-maze into the cup, and the ants were allowed to walk upwards one at a time and choose one arm before they were removed from the Y-maze. We conducted 10 trials, each with a different *F. rufibarbis* colony. For *T. caespitum*, four of seven colonies were tested twice, resulting in 11 trials in total. Data were analyzed as above, using an LME with Gaussian error distribution with 'colony ID' as a random factor.

To check whether ant choice was affected by the different experimental set-up (no food offered, ants recently separated from the nest), we conducted additional experiments with cuticular hydrocarbon extracts of *L. niger* workers, but tested them separated from the nest, in the same set-up as in the footprint assays. These assays were performed with seven colonies of *F. rufibarbis*, where we applied the extract of one *L. niger* worker in 25 µl of solvent to one arm and 25 µl of hexane to the other. Deviation from zero was then tested using an unpaired *t* test. Additionally, using LMs with Gaussian error distribution, we tested whether these results (cuticular hydrocarbon

extract of one worker, N = 7) resembled the results from the 'cuticular hydrocarbon extract assays' (cuticular hydrocarbon extract of five workers, N = 10). The simpler method revealed comparable results (unpaired t test: t_6 = 2.76, P = 0.033) and did not differ between the extracts with one and five workers (LM with Gaussian error distribution: $F_{1,15}$ = 0.03, P = 0.87). These assays were conducted in November 2013 and involved only colonies collected 2 weeks earlier.

All statistical analyses described above were calculated in R 3.0.2 (R Development Core Team 2013).

4.4. Results

Interspecific aggression

The five species differed strongly in their aggression in the interspecific assays (LME: $F_{4,45}$ = 8.08, P = 0.0001), resulting in a clear dominance hierarchy. *Lasius niger* and the slightly less aggressive *F. polyctena* (LME: t_9 = -1.90, P = 0.06) were the two most aggressive species in this dominance hierarchy. *Lasius niger* was significantly more aggressive than the remaining three species (LME: *T. caespitum*: t_9 = 3.04, P = 0.004: *F. rufibarbis*: t_9 = 4.10, P = 0.0002; *M. rubra*: t_9 = 5.21, P < 0.0001), whereas *F. polyctena* was more aggressive than *F. rufibarbis* (LME: t_9 = -3.15, P = 0.034) and *M. rubra* (LME: t_9 = 3.30, P = 0.002), but did not differ in aggression from *T. caespitum* (LME: t_9 = 1.13, P = 0.26; Fig. 4.1a). Based on significant aggression differences, we assigned the ants into two groups of dominants versus subordinates: (1) *L. niger* versus *T. caespitum*, *F. rufibarbis* and *M. rubra* and (2) *F. polyctena* versus *F. rufibarbis* and *M. rubra*, which served as the basis for the cuticular hydrocarbon and trail pheromone extract assays.

The species' ranking by aggression received from opponent species was inverse to the ranking by aggression inflicted. It increased from least aggression received in *L. niger* to highest aggression received in *M. rubra* (LME: $F_{4,146}$ = 7.54, P = 0.0001; Fig. 4.1b).

Analysis of the species-specific responses revealed that only *T. caespitum* (LME: $F_{3,27}$ = 2.97, P = 0.049) and *F. rufibarbis* (LME: $F_{3,27}$ = 4.54, P = 0.011) changed their aggression level depending on the species they interacted with (Appendix A4.1), exhibiting low aggression towards the dominant *L. niger*, but high aggression towards the subordinate

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M. rubra. A similar but nonsignificant pattern was detected in *F. polyctena* (LME: $F_{3,27}$ = 1.67, *P* = 0.19; Appendix A4.1). In turn, we found a similar but much stronger pattern for the aggression received (Appendix A4.2). *Formica polyctena* (LME: $F_{3,27}$ = 5.54, *P* = 0.004), *T. caespitum* (LME: $F_{3,27}$ = 3.07, *P* = 0.045) and *F. rufibarbis* (LME: $F_{3,27}$ = 5.00, *P* = 0.007) received different aggression levels from different opponent species, again in accordance with the dominance hierarchy. Workers of *M. rubra* and *L. niger* neither inflicted nor received different levels of aggression towards heterospecifics (both *P* > 0.39).
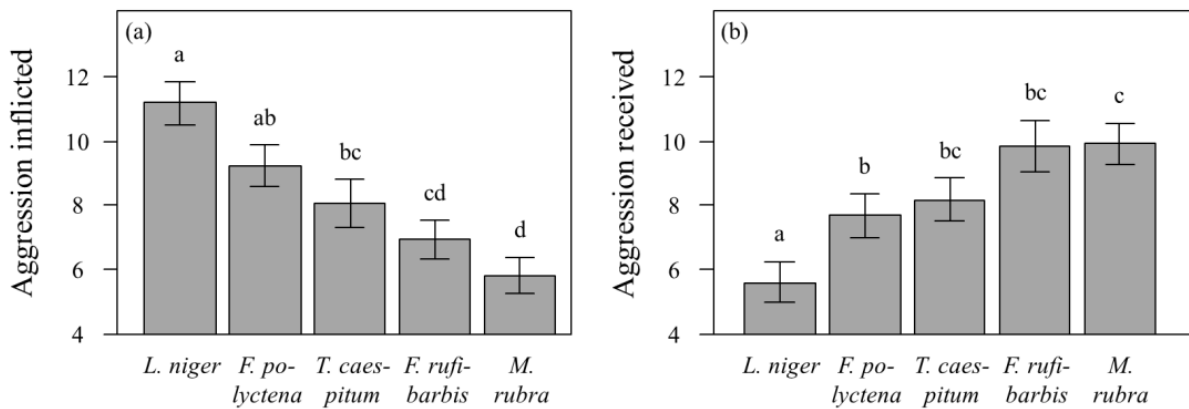

Figure 4.1: Interspecific aggression: mean number of attacks ± SE (a) inflicted on and (b) received from heterospecific ant species (*N* = 40/species). Different letters indicate significant differences based on linear models.

Cuticular hydrocarbons

The two subordinate species *T. caespitum* and *F. rufibarbis* both avoided areas covered with cuticular hydrocarbons of dominants (LMs: *T. caespitum*: t_9 = 4.43, *P* < 0.001; *F. rufibarbis*: t_9 = 2.14, *P* = 0.04 (*L. niger*) and t_9 = 4.43, *P* < 0.001 (*F. polyctena*); Fig. 4.2a). In contrast, *M. rubra* ignored both extracts of dominants (LMs: *L. niger*: t_9 = 1.28, *P* = 0.22: *F. polyctena*: t_9 = -1.14, *P* = 0.26; Fig. 2a).

Workers of the dominant species *L. niger* were neither attracted to nor avoided extracts of the three subordinate species (LM: $F_{3,27}$ = 0.94, *P* = 0.44; Fig. 4.2b). In contrast, workers of *F. polyctena* were attracted to areas covered with cuticular hydrocarbon extracts of both subordinate species tested (LMs: *F. rufibarbis*: t_9 = -3.20, *P* = 0.005; *M. rubra*: t_9 = -2.40, *P* = 0.028; Fig. 4.2b).
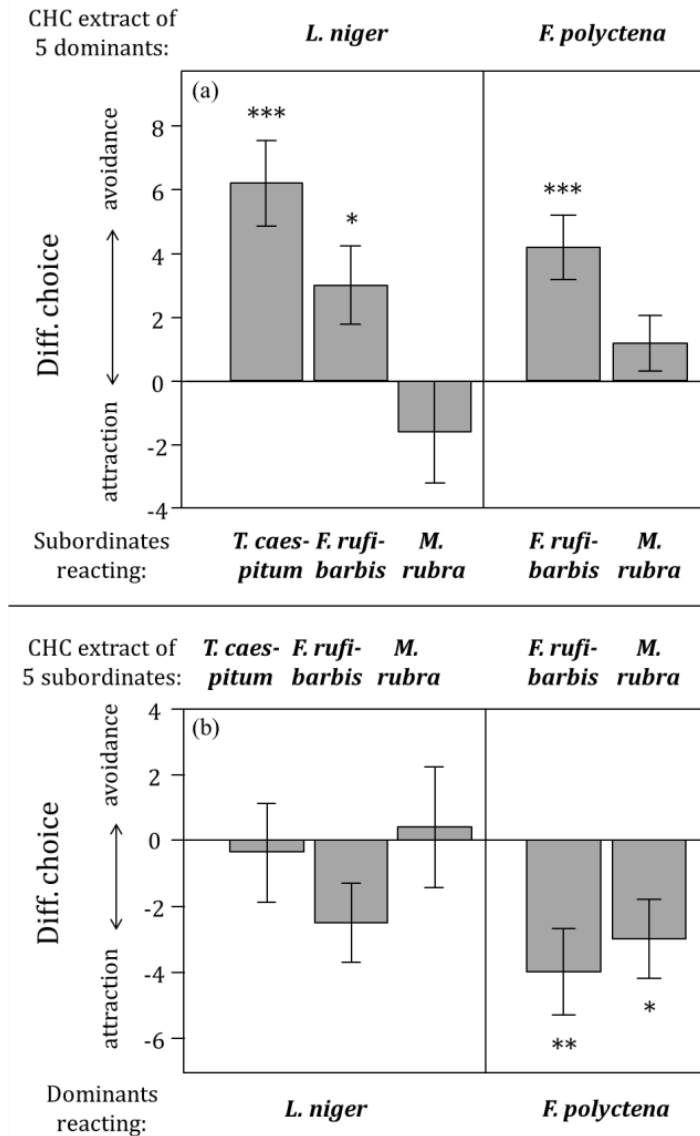
Figure 4.2: Cuticular hydrocarbon extracts: difference in choices ± SE of subordinate ant species between cuticular hydrocarbon extracts (CHC) of (a) the dominant *Lasius niger* or *Formica polyctena* and the respective control, and (b) vice versa. All species were represented by N = 10. Significance levels are based on linear models: *P < 0.05; **P < 0.01; ***P < 0.001.

Trail pheromones

Trail pheromone extracts of the dominant *L. niger* did not elicit avoidance or attraction in any of the three subordinate species (LM: $F_{3,27}$ = 1.02, P = 0.40; Fig. 4.3). Trail pheromone extracts of the second dominant *F. polyctena* showed an attraction in *M. rubra* workers (Fig. 4.3). However, because workers of *M. rubra* avoided following the

footprints of previous nestmates (see Footprints of nestmates below), we controlled their choices towards extracts for a potential footprint-following effect. Workers of *M. rubra* thus did not react towards trail pheromone extracts of the dominant *F. polyctena* (LME: $t_{1,9}$ = -1.75, P = 0.11) but avoided following previous nestmates (LM: $t_{1,9}$ = 2.34, P = 0.044). Similarly, workers of *F. rufibarbis* did not react to trail pheromone extracts of the dominant *F. polyctena* (LM: $t_{1,9}$ = -0.20, P = 0.85; Fig. 4.3).
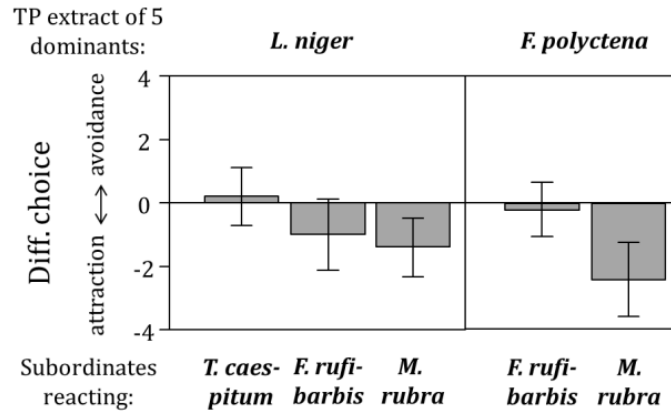

Figure 4.3: Trail pheromone extracts: difference in choices ± SE of subordinate ant species between trail pheromone extracts (TP) of the dominant *Lasius niger* or *Formica polyctena* and the respective control. All species were represented by N = 10. Significance levels are based on linear models: *P < 0.05.

Footprints of nestmates

Based on the order in which ants chose one or the other arm of the Y-maze, we found that workers of *M. rubra* avoided the footprints of the previous nestmate by choosing the opposite side (LM: t_9 = 3.63, P < 0.001). In the statistical analysis, we therefore controlled their choices from the extract assays for a potential footprint-following effect. This revealed that scouting foragers of *M. rubra* also avoided following the forerunner ant in all of the four different extract assays (LME: $F_{1,30}$ = 18.86, P = 0.0001). In contrast, all other species were unaffected by the footprints of their nestmates (LM: all P > 0.09; Fig. 4.4), revealing an independent choice.
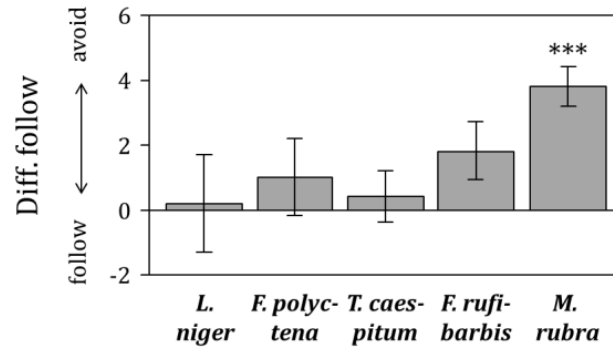
Figure 4.4: Footprints of nestmates: difference in following ± SE footprints of previous nestmate entering the Y-maze for all species investigated in this study. All species were represented by $N = 10$.

Footprints of dominant workers

In contrast to our findings in the experiments with cuticular hydrocarbon extracts, workers of the subordinate *F. rufibarbis* preferentially chose the arm on which workers of the dominant *L. niger* had walked before (LM: $t_9 = -3.90$, $P = 0.001$; Fig. 4.5). A similar trend was found in the second subordinate species, *T. caespitum* (LM: $t_6 = -1.81$, $P = 0.091$).
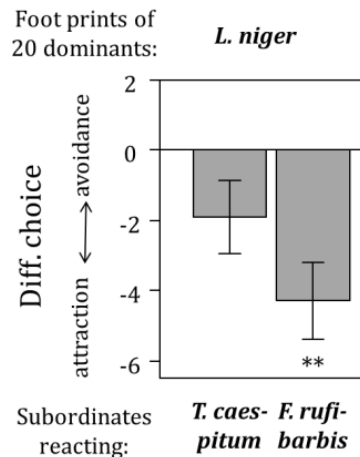
Figure 4.5: Footprints of dominant workers: difference in choices ± SE of subordinate ant species towards footprints of 20 individuals of the dominant *Lasius niger* and cuticular hydrocarbon extract (CHC) of only one specimen of *L. niger*. All species were represented by $N = 10$. Significance levels are based on linear models: **$P < 0.01$.

4.5. Discussion

Our study revealed that ant species are able to exploit olfactory cues unintentionally released by their syntopic competitors and include this information in their foraging

decisions. Their responses appear to be related to their rank in the community's dominance hierarchy and to olfactory cue intensity. Using Y-maze choice assays, we showed that subordinate ant species avoided cuticular hydrocarbon extracts but often approached footprints of dominant species, whereas dominant species either ignored or approached olfactory cues of lower-ranking ants. Moreover, subordinate species generally ignored the pheromone trails of the dominant species. Although we found some consistency in the reaction to chemical cues depending on dominance rank, not all species responded in the same way. For example, the lowest-ranking *M. rubra* did not react to heterospecific cues, but workers of this species avoided footprints of their nestmates in scouting situations.

Cuticular hydrocarbon extracts

Subordinate ant species (except for *M. rubra*) avoided areas covered with cuticular hydrocarbon extracts of behaviorally dominant species when foraging, probably to avoid direct resource competition. The high cue concentration of the cuticular hydrocarbon extracts indicated the recent presence of many dominant workers and thus a high probability of aggressive interspecific encounters. This can be important, as ant colony densities can be high in both the tropics and productive temperate sites (Steinmeyer et al. 2012). Indeed, local densities sometimes reached one colony each per m^2 of *L. niger*, *F. rufibarbis* and *T. caespitum* in our meadow site (our personal observations). Under these conditions, active avoidance of areas occupied by colonies of superior competitors has a high potential to reduce costs of aggressive encounters and thus could increase colony survival and food retrieval by workers. Such avoidance could be beneficial not only for foraging scouts, but also for founding queens when searching for suitable nesting sites (Sommer & Hölldobler 1995). By founding a colony away from the chemical cues of dominant species, young colonies would experience better starting conditions compared to the competitive situation in a 'bad neighborhood'. The resulting overdispersed spatial distribution of ant colonies has been found in ant communities both empirically and using simulations (Ryti & Case 1992). Similar interspecific competition avoidance also occurs in the golden spiny mouse, *Acomys russatus*, which avoids foraging when it detects the olfactory cues of a

superior competitor (Jacquot & Baudoin 2002). Indeed in mammals, which often heavily rely on their olfactory sense, chemical cues of competitors can induce behavioral responses as effectively as the competitor itself (Baudoin et al. 2013). The same holds true for the subordinate stingless bee *Melipona rufiventris* which avoids odor marks of its dominant competitor *Trigona spinipes* (Nieh et al. 2004). Many prey species try to circumvent predation by switching to antipredator behaviors when they detect reliable cues of their predators (Storm & Lima 2008, 2010). Our results similarly suggest that subordinate ant species use olfactory cues of dominant species to avoid costly competition.

The two dominant species showed diverging responses towards cuticular hydrocarbon extracts of subordinate ant species. While *F. polyctena* was strongly attracted to olfactory cues of subordinates, *L. niger* did not respond. *Formica polyctena* is a highly territorial ant species, which marks its territory with a colony-specific odor and defends it aggressively against inter- and intraspecific intruders (Hölldobler & Wilson 1977, 1990). Our results indicate that *F. polyctena* can not only detect the cuticular hydrocarbons of subordinate species, but also respond by orienting towards them. Comparable to olfactory hunting predators, which hunt their prey by following their chemical traces (Steidle & Loon 2003; Ylönen et al. 2003), this territorial ant appears to use subordinates' olfactory cues to localize competitors. This indicates that *F. polyctena* might prey on them as a food resource (Horstmann et al. 1982) or just kill them to prevent them from using their own resources (Hawes et al. 2013). In fact, dominant stingless bees also trace subordinates via their chemical cues to take over the food source (Nieh et al. 2004). In contrast, *L. niger*, the most aggressive species in our study, was unaffected by olfactory cues from subordinates. *Lasius niger* has been termed an 'encounter' species (Savolainen et al., 1989) which unintentionally marks its home range via footprints. Since the amounts released are very low, these markings are generally not colony-specific and may serve only as a general indicator of location quality. In contrast to *F. polyctena*, *L. niger* does not defend its home range against intruders and thus it is not surprising that this species does not respond to cues of sympatric ant species (Hölldobler & Wilson 1990; Cammaerts & Cammaerts 2000; Devigne & Detrain 2002; Lenoir et al. 2009).

Footprints of dominant workers

By testing subordinate ant species' choice between footprints of the dominant species *L. niger* and the respective control, we examined whether the response of subordinates also depended on cue concentration. Contrary to our assumption that subordinates would show a less pronounced response (i.e. ignore) to low quantities of dominants' olfactory cues, they were attracted to their footprints. In nature, low cue concentrations could indicate the presence of only a few dominant individuals or, alternatively, that they were present some time ago. A differential reaction towards high and low cue concentrations may be adaptive considering that the outcomes of aggressive encounters between social species such as ants depend not only on the species-specific rank in the hierarchy but also on their numerical dominance (Drescher et al. 2011). Our findings thus agree with Helfman's threat-sensitive hypothesis (1989) which states that a threat posed by competitors is much lower if only a few individuals are present. In fact, Seifert (2007) observed that single workers of the subordinate *F. rufibarbis* robbed food items from single workers of *L. niger*, the most aggressive species in our study. Similarly, the chances of winning a conflict increased for *T. caespitum* with decreasing numbers of *L. niger* opponents, because the latter species is superior in mobilizing and collective defense (Brian et al. 1966). The ability to subdue smaller groups of dominant species was not only found in interspecific interactions of social insects. For example, groups of coyotes, *Canis latrans*, can displace wolf, *Canis lupus*, packs if in superior number (Atwood 2006). Numerical abundance is hence crucial for the outcome of interspecific encounters. Our results suggest that ants may be able to gain information on the group size of dominant competitors by evaluating the intensity of olfactory cues. In a comparable manner, spider prey species use the intensity of chemical cues to assess the size, i.e. the predation risk, of predators (Persons & Rypstra 2001; Binz et al. 2014b).

Footprints of nestmates

The least aggressive species, *M. rubra,* did not change its behavior in response to heterospecific cues, but these ants avoided following their nestmate forerunners. This suggests that they can detect footprints of their nestmates and use this information in

their foraging or scouting decisions (e.g. Nonacs 1991), possibly to reach foraging areas not yet visited by nestmate scouts. Scouts of the ant *Leptothorax albipennis* were also suggested to deploy individual trails when evaluating the size of new nest sites (Mallon & Franks 2000). Moreover, the harvester ants *Pogonomyrmex maricopa* and *Pogonomyrmex californicus* use chemical markings during their search for food (Hölldobler 1970). These behaviors allow scouts to avoid redundant searches and thus increase the colony's foraging flexibility and thereby foraging success (Carroll & Janzen 1973). Still, our findings are, to our knowledge, the first to show that ants use olfactory cues to avoid nestmates when scouting for new food sources. Although this behavior should be beneficial for most ant species, it was only observed in *M. rubra*. In our study, *M. rubra* was most heavily attacked by the other species but was also the only species that did not respond to cues of dominant species. In many other studies, *M. rubra* has been reported to be a subordinate species (Vepsäläinen & Pisarski 1982; Savolainen et al. 1989; Cerdà et al. 2013) sensu the definition of Arnan, Gaucherel, and Andersen (2011), who specified that subordinate species would rather be ignored than attacked by dominant species. Thus, if *M. rubra* were ignored by dominant species in the field, it would not need to react to the presence of dominant species. However, other studies also reported that *M. rubra* is attacked by dominant species in the field (Vepsäläinen & Savolainen 1990). Dominance rank might thus depend on context for some species and vary with habitat type (Feener et al. 2008) or species composition (Brian et al. 1965). *Myrmica rubra*'s sister species *Myrmica ruginodis* avoids competition with *F. polyctena* by shifting its foraging strata to either higher shrubs or leaf litter. *Myrmica rubra* is also often found in the leaf litter (Savolainen & Vepsäläinen 1989) and might thus rely on heterogeneous vegetation to evade aggressive encounters with other species rather than their olfactory cues.

Trail pheromone extracts

Contrary to our expectations, none of the subordinate species responded to the pheromone trails of dominant species. Trail pheromones are not always species-specific (Attygalle & Morgan 1985) and it might therefore be hard to predict with whom

a worker would have to compete when following a heterospecific trail. Moreover, Akino and Yamaoka (2005) showed that *L. japonicus* needs footprints of their nestmates as a trail discrimination signal. Workers only followed trails in the presence of their colony-specific footprints but not trail pheromones alone. However, previous studies using pheromone extracts revealed that some ants can read heterospecific trail pheromones, even if they differ in chemical composition from their own pheromone (Menzel et al. 2010a, 2010b).

4.6. Conclusion

In conclusion, our study shows that footprints and cuticular hydrocarbons can be detected and interpreted by ants of other species and influence their foraging decisions. Our study demonstrates that hydrocarbon cues of dominants can be used by subordinate species to avoid interspecific competition. In turn, a dominant territorial species, *F. polyctena,* responded to cues of subordinates and oriented towards them, potentially to defend their territory from intruders. It seems likely that the ability to detect and respond to cues of other ant species is an important mechanism by which subordinate species reduce competition with dominants. Thus, responses to heterospecific cues are likely to have a strong impact on ant community structure. This includes the spatial distribution of foraging areas used by different species, but, if founding queens use heterospecific cues as well, it may also include the spatial distribution of colonies. Further studies are warranted to investigate why certain species ignore heterospecific cues and others vary their response depending on the cue concentration.

4.7. Acknowledgements

4.8. Appendix

Appendix A4.1: Interspecific aggression inflicted:. mean number of attacks ± SE inflicted among the five ant species (N = 40/species). Significance levels are based on linear models: *P < 0.05. Different letters indicate significant differences based on linear models.
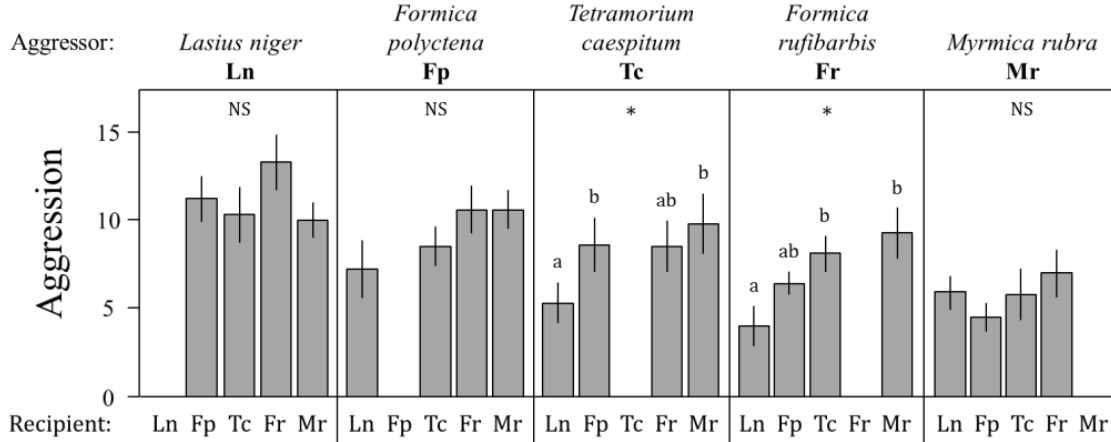
Appendix A4.2: Interspecific aggression received: mean number of attacks ± SE received among the five species (N = 40/species). Significance levels are based on linear models: **P < 0.01, *P < 0.05. Different letters indicate significant differences based on linear models.
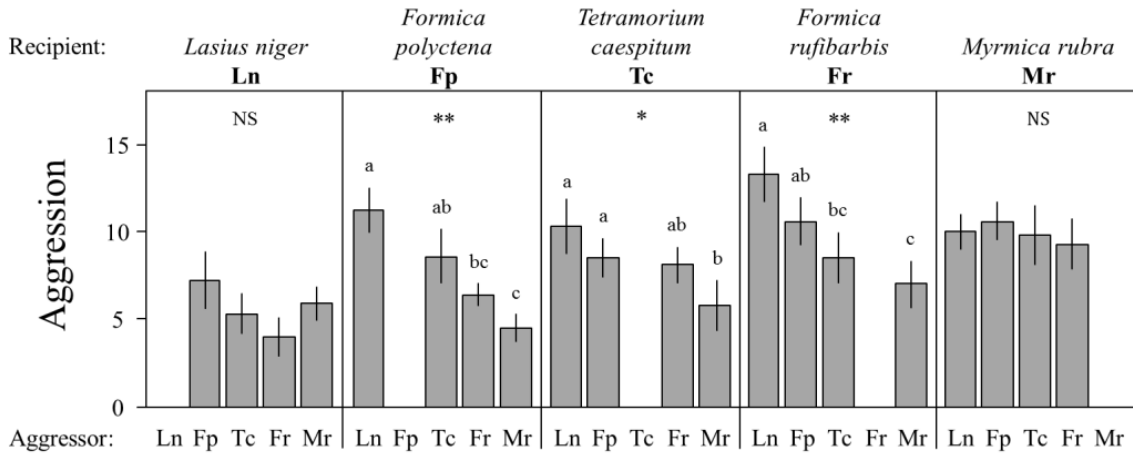
General discussion

My dissertation sheds light on the role of chemotactile cues in intra- and especially interspecific interactions among Central-European arthropod communities. In particular, we provide empirical evidence that olfactory cues can be used by several arthropods in favor of their own benefit. Moreover, we show that the resulting behavioral changes are species-specific and can depend on the trophic level, the dominance rank and the life-history traits of the species involved. In summary, the results of my dissertation show that it is of great importance to investigate the role of chemotactile cues in animal interactions as a basis for a better understanding of their ecological relevance and their potential effects on coexistence patterns and ultimately species diversity.

Behavioral trade-off in prey species

Not all investigated prey species showed behavioral responses to chemotactile cues of their potential predators. This can have two reasons: First, the potential prey species can't perceive the cues or obtain the information they might transmit. Spiders are generalist predators preying on a wide range of different arthropod prey species. Some of the investigated prey species might just suffer less from spider predation than others and have not evolved costly antipredator tactics towards chemotactile cues of rare encountered predators (Dawkins 1999). The potential resulting advantage for less abundant predators can thereby enhance their coexistence probability with more competitive or abundant predators (Matsuda et al. 1993, 1996). Second, other prey-specific adaptations could be more advantageous as defense against predation (Vet 1999). Although most arthropods primarily live in a chemical world (Greenfield 2002), they can also possess various other high-tuned senses like highly evolved eyes (Clemente et al. 2010) or extremely sensitive hairs (Tautz & Markl 1978), which may sometimes be the more efficient defense mechanism than olfaction. Crickets and cockroaches, for example, possess highly developed olfactory memories (Watanabe et al. 2003; Matsumoto & Mizunami 2005, 2006) but also air-sensitive

cerci (Camhi et al. 1978; Dangles et al. 2005). With their cerci, they can perceive approaching predators via air movements (Dupuy et al. 2011) and use it as a generalized defense against various different predators (Sih et al. 1998). Spiders therefore evolved hunting tactics to circumvent being detected by the cerci of their prey (Dangles et al. 2006). By responding to chemotactile cues of their spider predators, the investigated crickets (*Nemobius sylvestris*) and cockroaches (*Ectobius sylvestris*) thus mitigated the potential risk of spider predation by adapting a more predator-specific defense. Predatory wood ants (*Formica polyctena*), in contrast, follow escaping wood crickets with its constant speed without any success (author pers. obs.). This would explain the missing response to the chemotactile cues of wood ants as there is no need to evolve such tactic due to the generalized defense. We therefore assume arthropods to trade-off the investment into chemotactile cue induced responses if other, more generalized defensive reactions are sufficient to avoid predation or minimize possible incurred fitness costs. This apparent joint use of specialized and generalized antipredator behaviors in one prey species can also facilitate the endurance of a prey species with multiple different predators in the same habitat and thus ultimately stabilize their coexistence (Ikegawa et al. 2015). Moreover, experimental set-up was limited to study possible behavioral changes upon cue encounter. However, it cannot be ruled out that instead of changing measurable activity patterns, some of the prey species might rather, for example, change feeding habits (Bucher et al. 2014b) or increase hiding propensities (Schmitz 2008a). The overarching role of predator chemotactile cues for distinct arthropod prey species is therefore hard to predict by arena experiments only.

Predator cues transmit predator-specific information

As mentioned before, wood crickets can strongly profit from responding to spider chemotactile cues in order to avoid predation. However, behavioral responses to spider cues were not consistently freezing behaviors (no activity at all) as one would assume on the basis of previous studies (Persons & Rypstra 2001; Persons et al. 2001; Storm & Lima 2008, 2010) but manifested either as freezing or escape behavior (highly increased activity), depending on the respective predator species. Albeit only cues of

few spider species elicited significant behavioral changes in wood crickets, overall strength of cricket responses were highly correlated to spider-cricket body-size ratio. This means on one hand, that spider chemotactile cues bear the potential of transmitting information about the risk intensity posed by its emitter. On the other hand, it also assumes that wood crickets are able to assess the risk intensity and respond in accordance with Lima and Bednekoff's predation risk allocation hypothesis (1999). These assumptions could be underlined by the findings of distinct wood cricket responses towards cues of the same spider species but at different life stages, corresponding to their changing life history traits during ontogeny (Lenler-Eriksen 1969; Dangles et al. 2007). Furthermore, agreeing to the 'rare enemy effect' (Dawkins 1999), wood crickets showed higher escape propensities when facing cues of common spider species which implies that they have learned or adapted to cues of predators they often encountered in their life. This finding substantiates our previous assumption that chemotactile cue induced behavioral responses can promote a minority-advantage mechanism which favors the persistence of less abundant predators (Matsuda et al. 1993). Altogether, we can conclude that single arthropod prey species can include predators' chemotactile cues to increase personal fitness by accomplishing predator-specific antipredator behaviors. In contrast to generalized defenses that are effective against a wide range of predator species (e.g. cerci stimulation), predator-specific antipredator behaviors can reduce competition between predators eventually facilitates their coexistence (Matsuda et al. 1996; Kondoh 2007). We thus summarize that chemotactile cues can play an important role in maintaining species diversity due to inducing predator-specific antipredator behaviors in prey species.

Predator's advantage of prey chemotactile cues

Predators can profit from prey-emitted chemotactile cues to increase their overall foraging success, their foraging success towards more preferred prey (Cárdenas et al. 2012) as well as foraging success over larger distances (Schatz & Hossaert-McKey 2010). However, as predators have to learn or adapt to chemotactile cues of their prey, they are thought to specialize on few prey species only (Longhurst & Howse 1978; Cárdenas et al. 2012), and generalists might therefore not be able to evolve foraging

strategies on the basis of prey chemotactile cues. In fact, Schatz and Hossaert-McKey (2010) could already show that the two generalist tropical ant species *Crematogaster sp.* and *Oecophylla smaragdina* can use prey chemotactile cues for prey localization. Both ant species learned to exploit odors emitted by receptive figs and two closely related fig wasp species (both: Agaonidae, Hymenoptera) that are associated to the figs. Nevertheless, their potential of detecting cues emitted by taxonomically distinct prey species remains unclear. To our knowledge, workers of *Formica polyctena* are the first generalist arthropod predators that have been shown to exploit chemotactile cues of two taxonomically distinct prey species: the nursery web spider *Pisaura mirabilis* (Pisauridae, Araneae, Arachnida) and the wood cricket *Nemobius sylvestris* (Gryllidae, Orthoptera, Insecta). They changed their activity comparable to the prey-search behavior *sensu* Weier and Feener (1995) and the 'success motivated search' *sensu* Vinson (1977) when confronted with chemotactile cues of both prey species. Moreover, behavioral responses of the wood ant coincided with prey preference and aggression towards dead individuals of both prey species. We therefore assume that generalist arthropod predators like the wood ant bear the potential of olfactory prey localization even if prey species are taxonomically distinct. This could be a further explanation of the high predation efficiency of wood ants, which dominate and strongly govern wide parts of coniferous and mixed forests throughout Middle and Northern Europe (Savolainen & Vepsäläinen 1989; Seifert 2007). Furthermore, wood ants seem to be important ecosystem agents as they did not discriminate between prey species assuming their potential to reduce overall abundance of prey species. The resulting overall decrease in prey interspecific competition intensity might not only maintain species coexistence but also increase species diversity among sites (Ryberg et al. 2012). Moreover, they can also promote the coexistence with predators specialized on high quality prey because generalists also tolerate prey species of a low quality in times when the high quality prey is rare (Holt et al. 2013). Nevertheless, further investigations are needed to enlighten the overall occurrence of olfactory prey localization in generalist arthropod predators, as well as to determine the proximate cause/-s eliciting wood ant olfactory prey search.

Dominance-related cue exploitation in competitors

Interspecific competition is an important ecological mechanism that influences population dynamics and community structures (Gibb & Johansson 2011; Dhondt 2012). The resulting selection pressure can strongly shape species traits and facilitate co-existence. In ants, interspecific competition is particularly pronounced (Parr & Gibb 2012), as most ant species have to share resources due to widespread omnivory and shortage of nesting sites. The species involved thus structure into dominance hierarchies with dominant species aggressively displacing inferior ones. By exploiting chemotactile cues of their competitors, ants should thus strongly increase personal and colony fitness. In fact, competitively inferior species can circumvent dangerous and costly interferences by avoiding areas covered with chemotactile cues of highly aggressive dominant species. However, if cue concentrations were low, they got attracted to those areas. This indicates that inferior ant species might be able to quantitatively assess the current risk intensity when detecting cues from dominant species. This is comparable to the size- and ontogeny-related strength of antipredator behaviors observed in prey species (Persons & Rypstra 2001). By adapting their responses to the respective threat, inferior species can thus remain competitive with dominants whereas a general avoidance of cues from dominants would ultimately lead to a competitive exclusion of the inferior species. Behavioral responses of inferior species upon detecting chemotactile cues of dominants can thus play an important role in maintaining species coexistence. Yet, they did not respond to pure pheromone trails of dominant opponents, suggesting that among the investigated species, trail pheromones alone cannot transmit species-specific information about their emitters (Akino & Yamaoka 2005). Albeit *Myrmica rubra* was most inferior among the investigated species, it did not respond to any of the presented heterospecific cues. Nonetheless, it strongly avoided following the traces of their own colony members during scouting events, likely to avoid redundant searches in order to increase the colony's foraging flexibility and thus foraging success (Carroll & Janzen 1973). Of the two dominant species, only workers of the territorial wood ant *F. polyctena* got attracted to chemotactile cues of subordinates. Comparable to their prey tracing behavior mentioned above, they might use subordinates' cues to retain their established territory against intruders or even to prey upon them, too. By

eavesdropping on chemotactile cues, wood ants may additionally strengthen their supremacy in governing wide parts of coniferous and mixed forests throughout Middle and Northern Europe. In contrast, the second dominant species, *Lasius niger*, which only defends a small home range, showed no response to subordinate cues. In summary, we conclude that chemotactile cues can facilitate species coexistence among competitors by inducing adaptive responses in the species concerned. The ability to include chemotactile cues of competing opponents into behavioral decisions may thus represent an additional functional trait that promotes species-specific niche-differentiation and ultimately species richness (Chase & Leibold 2003).

Future directions

Further investigations of chemotactile cue induced antipredator behaviors

Because only few species showed behavioral changes in our arena experiments (Chapter 1), we were not able to thoroughly fill the current knowledge gaps about the overall occurrence, importance and strength of antipredator behaviors towards chemotactile cues of potential predators. Further experiments should thus concentrate on approaches that are easy to observe or to measure as some of the prey species might rather, for example, change feeding habits (Bucher et al. 2014b) or increase hiding propensities (Schmitz 2008a). However, arthropod activity can only be analyzed by specific tracking softwares which make the results hard to validate. Additional Y-maze assays or food preference tests may therefore be helpful in detecting other behavioral changes that could be more beneficial for the respective prey species when detecting predator cues. Further studies should also include a wider range of potential predator species like for example ants, mantids, assassin bugs or ladybugs to reveal the overall importance of chemotactile cues in shaping arthropod predator-prey relationships.

Proximate causes of predator cue exploitation

Wood ants showed undistinguishable behavioral responses to chemotactile cues of both prey species which coincided with our findings of equal prey preference and aggressiveness. However, the proximate cause inducing prey search behavior remains still unclear. Further studies are thus needed to find out if wood ants may bear the

ability to learn all the different cues from potential prey species, if they just respond to all non-nestmate cues encountered during foraging or if the spiders and crickets indeed share one or more specific cuticular hydrocarbons attractive to wood ants. Furthermore, a broader approach, including more generalist predators, would be beneficial for approving our findings.

Concentration-dependent responses among competitors
Subordinate ant species avoided high concentrations of dominants' cues whereas they got attracted to low cue concentrations. Additional Y-maze assays with graded cue concentrations might be helpful in verifying possible quantity dependent decisions in subordinates. Furthermore, because subordinate ants are often better and faster in discovering new food sources (Fellers 1987), dominants would highly benefit by eavesdropping on their trail pheromones. It would therefore be advantageous to also test responses of dominant species towards trail pheromones of subordinates.

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Appendix

Author contributions

Publi- cation	Original idea	Work planned	Experi- ments	Statistical analysis	Manus- cript draft	Manus- cript finalized
1	▬	▬	▬	▪	▪	▬
2	▬	▬	▬	▬	▪	▬
3	▬	▬	▬	▪	▪	▬
4	▬	▬	▬	▪	▪	▬

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Author affiliations

Curriculum vitae

Hellena Binz

Address:

Johannes Gutenberg-University Mainz
Institute of Zoology, Evolutionary Biology
Johannes von Müller Weg 6
55099 Mainz, Germany
E-Mail: hellena.binz@gmx.de

Nationality:	German
Date of birth:	10.09.1984
Place of birth:	Bühl/Baden, Germany

Academic studies:

04/2011 – 03/2014	PhD student in the project 'non-consumptive effects', University of Landau and University of Mainz, Germany
10/2007 – 10/2010	Study of biology with a main focus on animal ecology and sociobiology at the University of Würzburg, Germany
10/2004 – 07/2007	Study of biology at the University of Karlsruhe, Germany

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Work experience:

04/2014 – 08/2014	Field assistant, tier3 solutions GmbH, Leverkusen, Germany
03/2011 – 03/2014	Scientific assistant, University of Landau and Mainz, Germany
06/2008 – 10/2009	Working student, DFG Priority Project 1374 – Pollination networks, University of Würzburg, Germany
06/2006 – 09/2007	Working student at mouse facilities, Forschungszentrum Karlsruhe (FZK), University of Karlsruhe, Germany

Languages:

German (mother tongue) English (working language), Spanish (good in speech and writing), French (basic knowledge)

Soft Skills:

02/2013 – 12/2013   Mentee in the Ada Lovelace Mentoring Program for young female scientists of mathematics, informatics and natural sciences, University of Mainz, Germany.

Publications:

Binz H., Kraft E.F., Entling M.H. and Menzel F. (in prep.). Behavioral response of a generalist predator to chemotactile cues of two taxonomically distinct prey species.

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Binz H., Bucher R., Entling M.H. and Menzel F. 2014. Knowing the risk: crickets distinguish between spider predators of different size and commonness. *Ethology* 120:99–110.

Scientific talks (first author is presenting author):

Binz H., Foitzik S., Staab F. and Menzel F. (2013). Hierarchy-dependent non-consumptive effects in ants. *2nd Global Conference on Entomology, Kuching, Sarawak, Borneo, Malaysia*.

Bucher R., **Binz H.**, Menzel F. and Entling M.H. (2013). Fitness consequences of antipredator behavior in crickets driven by spider cues. *19th International Congress of Arachnology, Kenting National Park, Taiwan.*

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Binz H., Bucher R., Entling M.H. and Menzel F**.** (2012). Antipredator behavior of the wood cricket *Nemobius sylvestris* varies among distinct spider species and their biological traits. *105th Annual Meeting of the Zoological Society of Germany (DZG), Konstanz, Germany*.

Bucher R., **Binz H**., Menzel F. and Entling M.H. (2012). Are insects afraid of spiders? Occurrence and importance of antipredator behavior. *27th European Congress of Arachnology, Ljubljana, Slovenia.*

Bucher R, **Binz H.**, Menzel F and Entling M.H. (2012). Are insects afraid of spiders? Occurrence and importance of antipredator behavior. *42nd Annual Meeting of the Ecological Society of Germany, Austria and Switzerland, Lüneburg, Germany.*

Poster presentations (first author is presenting author):

Binz H., Schulze C.H. and Linsenmair K.E. (2012). Effects of topography on forest butterfly assemblages in the Pacific Lowlands of Costa Rica. *Annual Meeting of the Society for Tropical Ecology (GTÖ), Erlangen, Germany.*

Binz H., Bucher R., Entling M.H. and Menzel F. (2011). Do Insects smell their enemies? Searching for Non-Consumptive Effects. *41th Annual Meeting of the Ecological Society of Germany, Austria and Switzerland, Oldenburg, Germany*.

Bucher R., **Binz H.**, Menzel F. and Entling M.H. (2011). Do insects smell their enemies? Searching for Non-consumptive effects. *26th European Congress of Arachnology, Sede Boqer Campus, Israel.*

Declaration/Versicherung

Für das Gesuch um Zulassung zur Promotion in den Fachbereichen 17 - 22 der Johannes Gutenberg-Universität Mainz.

Ich, Hellena Binz, geboren am 10.09.1984 in Bühl (Baden), versichere gemäß § 11, Abs. 3d der Promotionsordnung vom 22.12.2003, dass

- ich die heute als Dissertation vorgelegte Arbeit selbst angefertigt und alle benutzen Hilfsmittel (Literatur, Apparaturen, Material) in der Arbeit angegeben habe.

- ich die jetzt als Dissertation vorgelegte Arbeit nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht habe oder hatte.

- ich weder die jetzt als Dissertation vorgelegte Arbeit noch Teile einer Abhandlung bei einer anderen Fakultät bzw. einem anderen Fachbereich als Dissertation eingereicht hatte

Mainz, den 10.11.2014   (Hellena Binz)

Acknowledgements