The role of predator-prey naïveté for the invasion success of lady beetles –

A comparison of species interactions across two continents

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Abstract

According to the predator-prey naïveté hypothesis, a lack of co-evolutionary history between native prey and non-native predators can result in missing predator recognition and an inefficient antipredator response of prey. These mechanisms can also shape non-native predator-predator interactions. Predator-prey and predator-predator naïveté can contribute to the invasion success of non-native predators. To gain a better understanding of the underlying mechanisms of naïveté in insects, we examined the interactions between native and non-native lady beetle species (Coleoptera: Coccinellidae) and pea aphids *Acyrthosiphon pisum* (Hemiptera: Aphididae) as native prey and ants (Hymenoptera: Formicidae) as native predators. Experiments were conducted in Europe and North America to provide an intercontinental comparison of species-interactions. To test for predator-prey naïveté in the pea aphid, we compared avoidance behavior of aphids towards chemical cues of native and non-native lady beetles. Moreover, we quantified aphid consumption of lady beetles to assess their voracity. Predator-predator naïveté of ants was tested in interaction experiments between ants and native and non-native lady beetle species by assessing and comparing ant aggression and lady beetle reaction. Furthermore, we tested if cuticular chemical cues (cuticular hydrocarbons, CHCs) of lady beetles play a role in ant aggression and analyzed their composition to see if CHCs profiles are species-specific. Overall, we expected a weaker response of native aphids and ants towards non-native lady beetles and their cues compared to native lady beetles.

Our findings showed that pea aphids avoid chemical cues of native lady beetles. Moreover, we demonstrated missing cue avoidance behavior of pea aphids towards chemical cues of the non-native Asian lady beetle *Harmonia axyridis* only in Europe*,* but not in North America. On both continents, *H. axyrids* and *Coccinella septempunctata* were the largest lady beetle species and consumed the most aphids. The ant aggression experiments revealed differences in ant aggression as well as lady beetle reaction of native and non-native lady beetles on both continents. In Europe, the CHCs of lady beetle species were species-specific in their composition. Furthermore, we found that cuticular chemical cues of lady beetles contribute to ant aggression.

Missing cue avoidance behavior towards chemical cues of non-native *H. axyridis* indicates prey naïveté in Europe. As *H. axyridis* was introduced to North America a longer time ago, our findings suggest a rapid adaptation of avoidance behavior by pea aphids towards chemical cues of non-native lady beetles. Moreover, non-native lady beetles may benefit from reduced ant aggression or ant tolerance, which might facilitate the access to ant-tended aphids. Overall, predator-prey and predator-predator naïveté and, thus, associated predation and competition advantages of non-native predators might decrease over time. In contrast, a relatively large body size compared to native predators might benefit non-native predators and contribute to their establishment and invasion success on the long term.

Zusammenfassung

Die Hypothese der Räuber-Beute Naivität besagt, dass fehlende Koevolution zwischen heimischen Beutetieren und nicht heimischen Prädatoren dazu führen kann, dass Beutetiere den Prädator nicht erkennen und sich somit nicht effizient verteidigen können. Diese Mechanismen finden sich auch in Räuber-Räuber Interaktionen wieder. Die Naivität von heimischen Beutetieren und Prädatoren kann somit zum Invasionserfolg von nicht heimischen Räubern beitragen. Um ein besseres Verständnis der vorliegenden Mechanismen auf das Gebiet der Insekten zu erweitern, haben wir Interaktionen zwischen heimischen und nicht heimischen Marienkäfer-Arten (Coleoptera: Coccinellidae) und heimischen Erbsenblattläusen *Acyrthosiphon pisum* (Hemiptera: Aphididae) als Beutetieren und Ameisen (Hymenoptera: Formicidae) als Räubern getestet. Um einen transkontinentalen Vergleich zwischen den Arten-Interaktionen ziehen zu können, haben wir unsere Experimente in Europa und Nord Amerika durchgeführt. Um zu sehen, ob Räuber-Beute Naivität vorliegt, haben wir das Vermeidungsverhalten der Erbsenblattlaus gegenüber chemischen Signalstoffen zwischen heimischen und nicht heimischen Marienkäfern verglichen. Außerdem quantifizierenten wir die Prädation von Blattläusen durch Marienkäfer, um deren Gefräßigkeit zu erfassen. Um die Räuber-Räuber Naivität von Ameisen zu untersuchen, haben wir Interaktionsexperimente zwischen Ameisen und heimischen sowie nicht heimischen Marienkäfer-Arten durchgeführt und dabei sowohl die Aggression von Ameisen als auch die Reaktion von Marienkäfern erfasst und verglichen. Zudem haben wir uns die Rolle von kutikulären chemischen Signalstoffen (kutikulären Kohlenwasserstoffen, CHCs) von Marienkäfern in der Aggression von Ameisen näher angesehen und die Zusammensetzung dieser auf Artniveau bestimmt. Unsere Annahme bestand zusammenfassend darin, dass heimische Blattläuse und Ameisen schwächer auf nicht heimische Marienkäfer und ihre Signalstoffe im Vergleich zu heimischen Marienkäfern reagieren.

Unsere Ergebnisse demonstrierten, dass Blattläuse chemische Spuren von heimischen Marienkäfern vermeiden. Außerdem zeigten wir, dass Singnalstoffe des nicht heimischen Asiatischen Marienkäfers *Harmonia axyridis* zwar von Blattläusen in Europa, jedoch nicht von Blattläusen in Nord Amerika vermieden werden. Auf beiden Kontinenten waren *H. axyridis* und *Coccinella septempunctata* die größten Marienkäfer-Arten und fraßen die meisten Blattläuse. In den Ameisen Aggressionsexperimenten stellten wir fest, dass sich die Aggression von Ameisen sowie das Verhalten von Marienkäfern zwischen heimischen und nicht heimischen Marienkäfern auf beiden Kontinenten unterscheidet. Die Zusammensetzung der CHCs von Marienkäfern sind in Europa artspezifisch. Außerdem demonstrierten wir, dass kutikuläre chemische Signalstoffe zur Aggression von Marienkäfern beitragen.

Ein fehlendes Vermeidungsverhalten gegenüber chemischen Signalstoffen von *H. axyridis* in Europa weist auf Räuber-Beute Naivität hin. Da die Einführung von *H. axyridis* in Nord Amerika länger zurück liegt, deuten unsere Ergebnisse darauf hin, dass möglicherweise eine Adaptation des Vermeidungsverhaltens von *A. pisum* auf die chemischen Signalstoffe von

nicht heimischen Marienkäfern vorliegt. Zudem ist es möglich, dass heimische Marienkäfer-Arten von einer niedrigeren Ameisenaggression oder „Ameisentoleranz“ profitieren, welches den Zugang zu Blattläusen, welche von Ameisen betreut werden, erleichtern könnte. Zusammenfassend können die Räuber-Beute und die Räuber-Räuber Naivität und die damit verbundenen Prädations- und Wettbewerbsvorteile von nicht heimischen Prädatoren mit zunehmender Zeit schwinden. Im Vergleich dazu könnte eine große Körpergröße in Relation zu heimischen Prädatoren langfristig zur Ansiedlung und zum Invasionserfolg von nicht heimischen Marienkäfer-Arten beitragen.

CHAPTER 1

General introduction

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Biological invasions and biotic interactions

Biological invasions are among the main drivers of biodiversity loss (Vitousek et al.1996, Nentwig 2008, Galiana et al. 2014). The invasion process by non-native species can be classified as following: arrival, establishment, spread and impact in the invaded range (Lodge 1993). Accordingly, a non-native species is considered invasive, if it causes major ecological, environmental or economic impacts in the invaded range (Hufbauer & Torchin 2008). In particular, non-native predators can have tremendous effects on populations, communities and ecosystems (Blackburn et al. 2004, Salo et al. 2007, Vitousek et al. 2008). Research on biotic interactions of non-native predators and the invaded community contributes to a comprehensive understanding of predator invasion success (Blossey & Nötzold 1995, Torchin et al. 2003, Torchin & Mitchell 2004, Hufbauer & Torchin 2008, Sih et al. 2010). In predator-prey interactions, predators cause density-mediated effects by reducing prey population densities through consumption (consumptive effects) (Murdoch et al. 2003). Thus, predators exert a strong selection pressure on prey, resulting in the evolution of antipredator behaviors (Lima & Dill 1990). Antipredator behaviors can be physiological, behavioral and/or morphological traits reducing predation risk and increasing prey survival (Kats & Dill 1998, Lima & Dill 1990). The costs of antipredator behaviors can adversely affect growth, fecundity and/or survival of prey and are referred to as non-consumptive effects of predators (Abrams 1995). Similar to density mediated effects, these trait-mediated effects can reduce prey population densities (Werner & Peacor 2003, Preisser et al. 2005). The concept of trait-mediated effects can similarly be applied to intraguild interactions between predators, possessing traits involved e.g. in the prevention of competitive encounters (Mestre et al. 2014). Moreover, both density- and trait-mediated effects can indirectly affect adjacent trophic levels (Terborgh & Estes 2010, Ohgushi et al. 2012). Research of non-native predator-prey as well as intraguild interactions can reveal density- and trait-mediated effects of non-native predators and therefore contribute to a better understanding of predator invasion success.

Predator-prey and predator-predator naïveté

In non-native predator-prey interactions, naïve prey might fail to recognize a non-native predator, leading to missing, inappropriate or insufficient antipredator behavior, due to a lack of co-evolution (Cox & Lima 2006, Banks & Dickman 2007). Specifically, prey naïveté is divided into three levels: Level 1 naïveté refers to prey that lacks recognition of the non-native predator and consequently shows no antipredator behavior. Level 2 naïveté of prey implies recognition of the non-native predator as thread but an inappropriate antipredator response. Level 3 naïveté, describes prey, which recognizes and responds to the non-native predator by adopting antipredator behaviors, which are insufficient due to superior strategies of the non-native predator (Banks & Dickman 2007, Carthey & Banks 2014). Level 1 naïveté indicates that costs of antipredator behaviors in naïve prey are low, resulting in low non-consumptive effects of non-native predators on prey populations. However, ineffective antipredator behaviors of naïve prey towards non-native predators might lead to higher predation, causing high consumptive effects on prey populations (Sih et al. 2010). In contrast, Level 2 and 3 naïveté would result in high consumptive, as well as non-consumptive effects (Carthey & Banks 2014). Thus, lacking or failing antipredator responses by native prey can enable non-native predators to have higher consumptive effects than native predators. The consumptive advantages of non-native predators can facilitate the establishment, help to outcompete native predators and result in higher non-native predator population densities (Dickman 1996). A rapid increase in non-native predator numbers, however, can lead to a 'boom-bust' pattern, where prey numbers crash, followed by a crash in predator numbers or dispersal of the non-native predator into non-invaded areas (Simberloff & Gibbons 2004, Sih et al. 2010). Similarly, to predator-prey interactions, native predators or parasitoids might fail to recognize or appropriately respond to non-native predators, due to unknown predator cues (predator naïveté). In that case, non-native predators might benefit from released enemy pressure (low consumptive effects of native top predators) by existing, native top predators (Enemy release hypothesis Torchin et al. 2003, Torchin & Mitchell 2004). This can manifest in increased competitive abilities of non-native predators, due to reduced resource allocation to antipredator behaviors (reduced non-consumptive effects of the non-native predator) (EICA hypothesis, Blossey & Nötzold 1995). In addition, non-native predators might spend more time on foraging, instead of engaging in predator-predator interactions (Sih et al. 2010). In the case of cue dissimilarities between native and non-native predators, the advantage of released enemy pressure and increased competitive abilities, contributes to the non-native predator's ability to outcompete native predators. The benefits of predator-naïveté do not only involve intraguild interactions involving predation, but can also be applied to interference interactions (excluding predation) between native and non-native predators (Bucher et al. 2014). Thus, non-native predators benefit from a double advantage of prey- as well as predator-naïveté in the invaded range. This 'novelty advantage' in naïve prey- and naïve predator-interactions can facilitate the invasion success of non-native predators (Sih et al. 2010). Naïveté research is dominated by invasions on islands and freshwater ecosystems, specifically driven by the fundamental impact of some introduced predators on naïve prey in these isolated systems (Cox & Lima 2006, Blackburn et al. 2004). The role of naïveté among terrestrial arthropods lacks attention and requires research particularly addressing naïveté of the native community towards non-native insect predators (Cox & Lima 2006).

Predator recognition and antipredator response of prey

Sensory predator cues can serve as information for prey to assess predation risk (Lima & Dill 1990, Lima 1998, Hermann and Thaler 2014). Although a predator releases information in the form of specific signals to an intended receiver, prey can evolve recognition of these predator cues to reduce predation risk (Wyatt 2014). There are a variety of sensory cues, such as visual, auditory or chemical cues, differing in spread and persistence (Acharya & McNeil 1998, Chivers & Smith 1998, Sih et al. 2010). For example, visual cues during encounters can signal immediate predation risk, while chemical cues indicate traces of predators, persisting in the environment as indicators of predator presence (Kats & Dill 1998, Bytheway et al. 2013). Predator cues require detection and recognition of prey to reduce predation risk (Lima & Dill 1990). Successful recognition requires the perception and comparison of the cue to an internal recognition template (Payne et al. 2004, Sherman, Reeve & Pfennig 1997). Threshold-dependent matches between the internal template and the perceived cue can mediate recognition (Sturgis & Gordon 2010, Sherman, Reeve & Pfennig 1997, Blumstein & Bouskila 1996). In coevolved predator-prey interactions, prey can develop perceptual, behavioral and cognitive adaptations to recognize predator cues, driven by the selection pressure of predation (Payne et al 1984, Lima 1998, Carthey & Banks 2014). However, a lack of co-evolution of native prey and- non-native predators can result in a mismatch of non-native predator cues and the internal recognition template of native prey. This can explain failed recognition and consequently lack of antipredator behavior of naïve prey towards non-native predators (Payne et al. 2004). The novelty of a non-native predator is mainly driven by the 'degree of mismatch' of the non-native predator cue and the native prey's recognition template (Payne et al. 2004, Carthey & Banks 2014). Accordingly, the 'degree of similarities' between native and non-native predators can explain variation in prey response. The more similar a non-native predator compared to the native predator community, the more likely that prey recognizes and induces antipredator behaviors (Sih et al. 2010). Different approaches for the assessment of similarities can be considered, for example, taxonomic similarities. However, taxonomic similarities can fail to comply with functional or mechanistic similarities (Chalcraft & Resetarits 2003). Thus, Sih et al. (2010) propose to assess similarities at predator-prey interaction levels, which are divided into encounter, detection/recognition, and response. First, predator-prey encounters require spatio-temporal co-occurrence in activity of predator and prey (Preisser et al. 2007), suggesting a comparison of overlapping activity pattern of native and non-native predators (Sih et al. 2010). On detection and recognition level, Sih et al. (2010) suggest the 'cue similarity' hypothesis. Based on this hypothesis, the degree of cue similarities between native and non-native predators mediate differences in native prey response. Not only cue similarities, but also the specialization of predator cue recognition, in particular the usage of general and/or specific predator cues to assess predation risk, substantially influences prey response to non-native predators (Payne et al. 2004). General predator cues can lead to an over-estimation of predation risk for example, large moving objects can be non-predacious but still elicit an antipredator response (Dill 1974, Sih 1986). Hence, recognition of prey based on general predator cues, might enable prey to recognize non-native predators (Payne et al. 2004, Sih et al. 2010). In particular, general cues shared by native and non-native predators may lead to recognition of non-native predator cues by prey without prior co-evolutionary history. In contrast prey might use specific predator cues, for example, species-specific chemicals of predators used for intra-and interspecific signaling (Boughman 2002, Smadja & Butlin 2009, Carthey & Banks 2014). Specific cues used by prey to recognize native predators might lead to Level 1 naïveté towards non-native predators, due to a lack of recognition of specific, novel predator cues (Carthey & Banks 2014). Prey might use specific cues for recognition of native predators signalizing an elevated predation risk, increasing the chances of survival and reducing the costs for unnecessary response (Lima & Bednekoff 1999, Carthey & Banks 2014). In addition, prey can use a combination of multiple cues for predator recognition, for example chemical and visual cues from a specific predator (Amo et al. 2004). After the detection and recognition of predators, appropriate prey response via antipredator behaviors can reduce predation risk and ensure survival of prey (Kats & Dill 1998, Lima & Dill 1990). Prey might have evolved multiple antipredator behaviors, which can vary depending on predation risk and context (Endler 1991, Binz et al. 2014). Prey response can be a general and/or specific antipredator behavior and play a crucial role for the understanding of non-native predator effects on native communities (Lima 1992, Schoeppner & Relyea 2005, Brilot et al. 2012). A notable example of a coevolved specialized response is the adaptation of shell properties (eg. shell-thickness) by gastropods to reduce shell crushing by crab predators (Freeman & Beyers 2006). Predation risk from multiple predators, however, can result in general antipredator behaviors, being effective towards a wide range of predators. Increased vigilance and decreased movement activity are examples for general predator behaviors (Endler 1991). In contrast to specific antipredator behaviors, general antipredator-behaviors are more likely to be effective against non-native predators and therefore level 2 and 3 naïveté are less likely to occur in native prey (Banks & Dickmann 2007, Carthey & Banks 2014). Thus, prey responding with specific antipredator behaviors to a particular native predator are prone to naïveté (Level 2 or 3), when confronted with non-native predators (Carthey & Banks 2014). Overall, the degree of cue specificity (general versus specific cues) used by native prey and the mode of antipredator behaviors by prey (general versus specific response), in combination with similarities between native and non-native predators might explain why some native prey effectively respond to non-native predators, while others fail (Sih et al. 2010).

Lady beetles

The family Coccinellidae belong taxonomically to the order Coleoptera. Within the subfamily Coccinellinae, the majority of species are predacious and preferably consume aphids, referred to as aphidophagous lady beetle species (Gordon 1985, Hodek & Honěk 1996). Aphidophagous lady beetles have been globally redistributed for biological control, providing a valuable ecosystem service as predators of numerous agricultural pest species (Harmon et al. 2007, Obrycki & Kring 1998, Stern et al. 1959). Among them, the multicolored Asian lady beetle *Harmonia axyridis* (Pallas) is of Asian origin, native to China, Japan, Korea, Mongolia and Siberia (Dobzhansky 1933, Kuznetsov 1997, Brown et al. 2007). *Harmonia axyridis* has established beyond its native range, due to intentional and unintentional introductions (Day et al. 1994, Brown et al. 2011). In North America, *H. axyridis* was initially introduced in 1916 (Gordon 1985), followed by augmented releases in the 1970s and 1980s (Tedders & Schaefer 1994, Koch & Galvan 2007) and the first reported establishment in 1988 (Chapin & Brou 1991). *Harmonia axyridis* was not only a promising non-native biological control agent of aphids in North America, but also in Europe (Brown et al. 2007) introduced to France in 1982 (Iperti & Bertrand 1982). Initially quarantined, field experiments were conducted from 1990, followed by the first commercial field releases in 1995 (Brown et al. 2007). First establishments of *H. axyridis* were reported in the late 1990s and this species rapidly spread throughout Europe. *H. axyridis* is eurytopic, inhabiting arboreal and semi-arboreal habitats and meadows, heathlands, riparian zones, reedbeds and agricultural systems (Brown et al 2007). It is polyphagous with a broad dietary range, feeding not only on many aphid pest species but also non-target species and pollen, nectar and fruit (van Lenteren et al. 2007, Roy et al. 2016, Koch 2003). Although, *H. axyridis* is considered bivoltine in its native range (Osawa 2000), in North America (Koch and Hutchison 2003), and Europe (Ongagna & Iperti 1993), up to four to five generations per year were documented (Wang 1986, Katsoyannos et al. 1997, Koch 2003). In predator-predator interactions, *H. axyridis* is a successful intraguild predator of native coccinellids in its invaded range (Snyder et al. 2004, Pell et al. 2008). This is beneficial in dietary terms, but also reduces competition on shared resources (Yasuda et al. 2004, Dixon 2000). Roy et al (2006) labeled *H. axyridis* as the ‘most invasive lady beetle species on Earth’, due to its successful spread and establishment in many parts of the world and its adverse effects on invaded communities. In addition to *H. axyridis*, *Coccinella septempunctata* (Linnaeus) originally of Palearctic origin (Honěk & Hodek 1996), was introduced intentionally and accidentally to North America in 1956 (Day et al. 1994, Angalet and Jacques 1975). The earliest documented establishment dates back to 1973 (Angalet and Jacques 1975). *Coccinella septempunctata* is polyphagous, primarily feeding on aphids, but also on nectar and pollen (Ricci et al. 2005). Compared to *H. axyrids, C. septempunctata* uses fewer aphid species as essential food source and seems to be less polyphagous (Koch 2003, Hodek & Michaud 2008). Similar to *H. axyridis,* *C. septempunctata* is eurytopic, inhabiting arboreal and herbaceous habitats, including grasslands, fields and orchards (Honěk & Hodek 1996). Voltinism in *C. septempunctata* ranges from a univoltine cycle to a less common facultatively polyvoltine cycle in central Europe (Hodek 1966) and this species shows a similar heterogenetic voltinism pattern in North America (Obrycki & Tauber 1981, Angalet et al. 1979). *Coccinella septempunctata* can dominate as intraguild predator over some native coccinellids in its invaded range in North America (Snyder et al. 2004, Tumminello et al. 2015). Overall, *C. septempunctata* is classified as invasive species in North America, due to its abundance, widespread distribution and dominance in trophic-interactions, which negatively affects the native community (Eliott et al. 1996). Additionally, to *C. septempunctata* and *H. axyridis*, the Palearctic *Hippodamia variegata* (Goeze) has been released for biological control of aphids in North America from 1957 (Ellis et al. 1999), became established in 1984 (Gordon 1987) and is not considered as invasive, so far. *Harmonia axyridis*, *C. septempunctata* and *H. variegata* have been initially released for the biological control of aphids beyond their native range to reduce the detrimental effect of aphid pests on crops (Angalet et al. 1979, Tedders and Schaefer 1994, Ellis et al. 1999, Stern et al. 1959).

Aphids

The family Aphididae belongs taxonomically to the Order Hemiptera. Aphids are phytophagous, sap-sucking insects with significant economic importance, when gaining pest status in agricultural environments (Blackman & Eastop 2017). The pea aphid *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) is listed among the 15 aphid species of most agricultural importance (Blackman & Eastop 2017). As primary host plants *A. pisum* feeds on plants of the family Fabaceae and can transfer more than 30 viruses, causing significant economic damage on crops (Blackman & Eastop 2017). Originally a Palearctic species, *A. pisum* has spread globally since the late nineteenth-century (Thomas 1878). *Acyrthosiphon pisum*, consist of multiple genetically differentiated biotypes, specialized on different host plants (Peccoud et al. 2009, Via 1999). The biology and host-plant preference of globally distributed *A. pisum* populations may have diverged from the original European population (Blackman & Eastop 2017). In Western Europe, Peccoud et al (2009) found at least 11 sympatric populations of *A. pisum* associated with different host plants. Additionally, in North America, *A. pisum* populations specialized on red clover and alfalfa are genetically distinct and demonstrated varying preference and fitness on specific host plants (Caillaud & Via 2000, Via 1999). The life cycle of the pea aphid consists of female, parthenogenetic generations, which alternate with sexual generations. Asexual reproduction alone can appear in some populations, especially in regions without cold winters. In spring and summers, asexual females produce clonal offspring.

The larvae undergo four molts and can develop as unwinged or winged asexually reproducing adults. Winged offspring development is induced by crowding or prenatal stress. Asexual reproduction ceases, when shorter autumn day lengths induce the production of unwinged sexual females and males. In pea aphids, the production of winged or unwinged aphids is genotype-depending. The sexual females are oviparous and produce overwintering eggs, after mating. In spring, the hatched aphids produce asexual, wingless females (Blackman & Eastop 1984, The International Aphid Genomics Consortium 2010).

Aphid-lady beetle interactions

In predator-prey interactions, aphids respond to direct predator contact, vibrations on plant caused by predators, alarm pheromones produced by a conspecific and chemical predator cues on the feeding side (Klingauf 1967, Montgomery & Nault 1977, Roitberg & Myers 1978, Clegg & Barlow 1982, Dixon & Argawala 1999, Ninkovic et al. 2013). *Acyrthosiphon pisum* developed numerous antipredator behaviors to respond to predators, such as kicking, stylet removal, walking away, and dropping from the plant (Roitberg et al. 1979). The production of an alarm pheromone with the primary component (E)-β-farnesene is an example for intraspecific signaling in aphids to alarm conspecifics of predators (Bowers et al. 1972, Kislow & Edwards 1972, Al Abassi 2000). After neighboring aphids detect the pheromone released by an attacked conspecific, they can respond in different ways, such as vigorous movements of leg and antennae, walking away or dropping from the plant (Dill et al. 1990). Predator cues induce aphid dispersal to sites with less predator pressure (Roitberg et al. 1979). Aphids can respond to interspecific chemical cues, such as lady beetle footprints of larvae, leading to the production of winged aphid morphs and dispersal to new feeding sides (Dixon & Argawala 1999). Recently, researchers discovered that aphids can respond to footprints of *C. septempunctata* by avoiding the present feeding side (Ninkovic et al. 2013). The chemical composition of these cues are species-specific, thus it remains unknown, if aphids can respond to chemical cues of various lady beetle species (Magro et al. 2010, Kosaki & Yamaoka 1996). While searching for suitable feeding sides, these chemical cues can indicate predation risk on the current site. Avoidance of sites with less predator pressure, might reduce survival costs and predation risk (Roitberg et al. 1979). Antipredator responses can be energetically costly, causing feeding interruptions and survival costs through the risk of death by desiccation or ground predators after leaving the present host plant (Roitberg & Myers 1979, Dill et al. 1990). Nelson (2007) demonstrated that predator-induced disturbances of *A. pisum*, resulted in loss of feeding time and reduced reproduction (non-consumptive effects), which increased with the frequency of antipredator response and costs associated with the response.

Ant-lady beetle interactions

Aphids feeding on phloem-sap, produce a carbohydrate-rich excretion referred to as 'honeydew'. Aphids excrete excess carbohydrates to balance the high carbohydrate/amino acid ratio of the ingested phloem sap with their dietary requirements (Wäckers 2000). Some aphid species can engage in mutualistic interactions with aphid-tending ant species (Hymenoptera: Formicidae) (Way 1963). These aphid-tending ants use the 'honeydew' produced by aphids as a food source and in return protect aphids from fungal pathogens (Nielsen et al. 2010) and predation and parasitism (Way 1963). As aphidophagous lady beetles and aphid-tending ants rely on aphids as food sources, they can engage in competitive interactions. Ant- lady beetle interactions are an example for intraguild interference, implying resource competition between spatio-temporally coinciding predators, in which direct predator interactions limit the access to a resource (Putman 1994). Intraguild interference can reduce the fitness of the subordinate predator by e.g. aggressive interactions (Eccard & Ylönen 2002). Interactions between lady beetles and ants can differ, depending on size, aggressiveness and density of tending ants, as well as size, behavior and capabilities of defensive behavior of lady beetles (Majerus et al. 2007). Cuticular hydrocarbons of parasitoid and predator species can serve as interspecific recognition cues, eliciting ant aggression (Pasteels 2007, Dettner & Liepert 1994). Cuticular hydrocarbons (CHCs) cover the external layer of the insect exoskeleton and primarily function as waterproofing agent protecting against desiccation (Lockey 1988). Various insects use CHCs as intra- and interspecific chemical communication (Durieux et al. 2012, Lang & Menzel 2011). Cuticular hydrocarbons, used for communication, are perceived as contact or short-range signals, due to their chemical properties (non- or semi-volatile long-chain hydrocarbons) (Howard & Blomquist 2005). As insects walk, they inevitably leave footprints behind, consisting of hydrocarbon droplets (Devigne & Detrain 2006). Ant species can detect and respond to footprints, using hydrocarbons as chemical cues to reduce the costs of competition (Wüst & Menzel 2017). The hydrocarbons of lady beetle footprints are species-specific and largely identical to cuticular hydrocarbons (Kosaki & Yamaoka 1996, Geiselhardt et al. 2011, Hemptinne & Dixon 2000). The CHCs of lady beetle footprints serve, for example, as oviposition deterring pheromone, preventing female conspecific to oviposit in the same patch (Doumbia et al. 1998). The CHC composition of closely related lady beetle species tend to be more similar compared to the CHCs composition of distantly related lady beetle species. Specifically, chemical cues within some lady beetle genera are more similar than between genera (Magro et al. 2010). It remains to be tested, if species-specific cuticular hydrocarbons of lady beetle species induce varying aggression in ant species and if ants are naïve to novel lady beetle cues, but respond to similar non-native lady beetle cues.

Aim of thesis

The predator-prey-naïveté hypothesis suggests that a lack of co-evolutionary history between non-native predator and native prey can lead to missing predator recognition and an ineffective or missing antipredator behavior (Diamond & Case 1986, Banks & Dickman 2007, Sih et al. 2010). This can similarly apply to predator-predator interactions, referred to as predator-predator naïveté (Bucher et al. 2014, Sih et al. 2010). **Non-native predators** might benefit from **predator-prey** and/or **predator-predator naïveté** in the new range, contributing to their invasion success (Sih et a. 2010, Carthey & Banks 2014). To date, there is a lack of studies targeting naïveté among terrestrial arthropods (Cox & Lima 2006). This thesis aims to shed light on the **mechanisms behind invasion success of lady beetles**, targeting predator-prey and predator-predator naïveté of native prey and predators based on the framework of Sih et al. (2010). We examined the interactions between native and non-native lady beetle species (Coleoptera: Coccinellidae) and pea aphids (Hemiptera: Aphididae) as native prey and ants (Hymenoptera: Formicidae) as native predators. Our first model system, representing predator-prey interactions, consists of **native and non-native lady beetle-aphid interactions** (**Chapter 2-4**). The second model system is an example for predator-predator interactions (i.e. intraguild interference) consisting of **native and non-native lady beetle-ant interactions** (**Chapter 5-6**). Our research was conducted in **Europe and North America**, allowing for an intercontinental comparison of species-interactions. The underlying findings are based on laboratory experiments and contribute to a basic understanding on mechanisms behind invasion success. Overall, our research can not only help to understand why some insect species become invasive while others fail, but additionally contribute to a better comprehension of **ecological impacts** of introduced insect species in the future.

Study species

Europe

In Europe our lady beetle species-set consisted of four native European lady beetle species, *Coccinella septempunctata* (Linnaeus)*, Adalia bipunctata* (Linnaeus), *Propylea quatuordecimpunctata* (Linnaeus), and *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) and non-native Asian *Harmonia axyridis* (Pallas). In the predator-prey interactions we used a native population of the pea aphid *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae). In the predator-predator interactions (intraguild interference) we used *Lasius niger* (Linnaeus) and *Myrmica rubra* (Linnaeus) as predators.

North America

In North America our lady beetle species-set consisted of three native North American lady beetle species *Coleomegilla maculata* (De Geer) *Coccinella novemnotata* (Herbst) and *Hippodamia* convergens (Guérin-Méneville), two non-native European lady beetle species *Coccinella septempunctata* and *Hippodamia variegata* and the non-native Asian lady beetle species *Harmonia axyridis*. The native and non-native lady beetle species of the genera Hippodamia and Coccinella are expected to bear similar cues (Magro et al. 2010) and are referred to as congeneric. We used a native population of the pea aphid *Acyrthosiphon pisum* as prey species. Moreover, we used *Lasius neoniger* (Emery) and *Myrmica americana* (Weber) as ant species for the predator-predator interactions.

Comparison of ladybeetle consumption and aphid avoidance

First, we conducted **leaf-choice experiments** and subsequently a **predation experiment** in Petri dishes in **Europe** (**Chapter 2**) and in **North America** (**Chapter 3**) resulting in two studies. In particular, we tested if native ***A. pisum*** **avoids chemical cues of** native and non-native **lady beetles** and subsequently can discriminate between them. Therefore, we set up a two-choice experiment with an untreated control and a treatment leaf bearing chemical cues of lady beetles. In this leaf-choice experiment, we quantified aphid leaf choice and subsequently compared it between lady beetle species. The preference for the control leaf indicated avoidance behavior of lady beetles. In Europe we expected missing avoidance behavior of *A. pisum* towards chemical cues of non-native *H. axyridis* and avoidance behavior towards chemical cues of the remaining four native lady beetle species (**Chapter 2**). In contrast to the European study, the **North American** study considered potential similarities between non-**native and native lady beetle species** by including species of the **same genera**. In North America we expected missing avoidance behavior of *A. pisum* towards chemical cues of non-native *H. axyridis*, intermediate avoidance behavior towards congeneric *H. variegata* and *C. septempunctata* (due to expected **cue similarities** to congeneric native species) and comparably strongest avoidance behavior towards chemical cues of native lady beetle species (**Chapter 3**). In a second Petri dish experiment we quantified aphid consumption of lady beetles. In Europe and in North America, we expected higher aphid consumption of the similairly large *H. axyridis* and *C. septempunctata* due to body-size related food demands compared to smaller lady beetle species. We additionally compared cue avoidance behavior of aphids and aphid predation of native *H. variegata* and *C. septempunctata* as well as non-native *H. axyridis* quantified in Europe, with a subset of quantified behaviors of the same species in North America. This served as a comparison of predator-prey interactions involving

H. variegata and *C. septempunctata* in the native European *versus* the non-native North American range, as well as a comparison of predator-prey interactions involving non-native *H. axyridis* in Europe *versus* North America.

Second, we conducted a more natural experiment on plants in **Europe** (**Chapter 4**), testing **dropping behavior** and **aphid consumption** and in a second experiment **plant-choice** of aphids. In the first experiment, we quantified initial dropping behavior of aphids from plants, expecting lower dropping rates in the presence of non-native *H. axyridis* compared to native European lady beetle species. In the same experiment we quantified aphid consumption, expecting higher consumption rates of *H. axyridis* compared to the remaining native lady beetle species. In the additional plant-choice experiment, we tested if native ***A. pisum*** **avoids chemical cues** of native and non-native **lady beetles** and subsequently discriminates between them. We set up a two-choice experiment with an untreated control and a treatment plant bearing chemical lady beetle cues. Aphid plant choice was quantified and subsequently compared between the different lady beetle species. The preference for the control plant indicated avoidance behavior of lady beetles. We expected missing avoidance behavior of *A. pisum* towards chemical cues of non-native *H. axyridis* and avoidance behavior towards chemical cues of coevolved native lady beetle species.

Ant aggression, ladybeetle reaction, and the role of chemical ladybeetle cues

Third, we conducted **ant aggression experiments** with **lady beetles** and additionally **dummies** (lady beetle elytra) bearing chemical lady beetle cues in Petri dishes in **Europe** (**Chapter 5**) and in **North America** (**Chapter 6**), resulting in two studies. In the first experiment, we quantified and subsequently compared **ant aggression** and **lady beetle reaction** of the different lady beetle species. In the second experiment we tested, if cuticular **chemical cues (cuticular hydrocarbons, CHCs) of lady beetles** play a role in ant aggression. We used lady beetle elytra as dummies bearing chemical cues (CHCs) of native and non-native lady beetles to quantify ant aggression. We manipulated the dummy elytra, obtaining three different treatments: control elytra, cue-treated elytra (cue-free elytra treated with chemical lady beetle cues) and cue-free elytra. We expected the control and cue treated elytra to elicit similar aggression strength compared to the cue-free elytra. In the European study, we expected in both experiments comparably higher aggression strength towards native lady beetles and cues and reduced aggression strength towards non-native *H. axyridis* and cues respectively. In **North America**, we expected comparably higher aggression strength towards native lady beetles and cues, intermediate aggression strength towards **congeneric** non-native *H. variegata* and *C. septempunctata* and cues (due to expected **cue similarities** to congeneric native species) and reduced aggression strength towards non-native *H. axyridis* and cues, respectively. We analyzed the chemical composition of CHCs using GC-MS in Europe, expecting species-specific **CHCs profiles** for each **lady beetle species**. The analysis of CHCs profiles in North America is expected to reveal cue similarities in the chemical composition of native and non-native species of the genera Hippodamia and Coccinella in North America and is not included in this thesis.

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CHAPTER 2

Predation and avoidance behaviour in aphid-ladybird interactions of native and invasive ladybirds in Europe

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Abstract

While detrimental effects of invasive predators on native species are well documented, we often lack a mechanistic understanding of the invasion success. Lack of prey avoidance behaviour can lead to higher consumption rates by invasive predators compared to native predators. This competitive advantage is expected to contribute to the invasion success of non-native predators.

We compared aphid consumption and cue avoidance behaviour of aphids between four native ladybird species (*Coccinella septempunctata*, *Adalia bipunctata*, *Propylea quatuordecimpunctata*, and *Hippodamia variegata*) and the invasive Asian ladybird *Harmonia axyridis*. The invasive *H. axyridis* and the native *C. septempunctata* consumed more aphids than the three smaller native ladybird species. In line with our expectations, aphids avoided leaves bearing cues of most native ladybird species but not of the invasive *H. axyridis*.

Our results indicate that body size rather than ladybird origin determined aphid predation rates. The lack of aphid avoidance behaviour towards cues of *H. axyridis* indicates that they were not able to recognize chemical cues of the invasive predator. Relatively large body size and the absence of cue avoidance in aphids might benefit the invasive *H. axyridis*, particularly in comparison to smaller native ladybird species. The absence of avoidance behaviour in aphids might lead to even higher predation rates of *H. axyridis* under more natural conditions.

Keywords: *Harmonia axyridis*, *Acyrthosiphon pisum*, naiveté hypothesis, prey naiveté, predator-prey interactions, invasive species, cue avoidance

Introduction

The increasing number of invasive species is a global threat to biodiversity and a major cause of species extinctions (Blackburn et al., 2004; Clavero & García-Berthou, 2005; Lowe et al., 2000). In particular, the establishment of non-native predators can lead to declines in prey populations and disturb predator-prey interactions (Paolucci et al., 2013), as invasive predators often inflict greater predation pressure on native prey populations compared to native predators (Paolucci et al., 2013; Salo et al., 2007). During co-evolution, prey species have often evolved detection mechanisms, allowing them to recognize a predator (e.g. chemical or visual cues), as well as avoidance and escape mechanisms (e.g. hiding, camouflaging, running, defence techniques) to reduce predation risk (Carthey & Banks, 2014; Sih et al., 2010). In this regard, chemical cues are of particular interest because they persist in environments and might inform prey about potential predation risk (i.e. landscape of fear) (Bucher et al., 2015; Laundré et al., 2010). If prey and predator do not share a co-evolutionary history, however prey may not be able to detect or appropriately respond to the predator's cues, leading to missing predator recognition and lacking or inefficient response behaviour (Sih et al., 2010). This concept is described by Cox and Lima (2006) as the naiveté hypothesis and has been demonstrated in several studies (Barrio et al., 2010; C. Brown & Warburton, 1999; Carthey & Banks, 2014).

For prey, the detection and recognition of predators before actual encounters is crucial for survival (Dawkins & Krebs, 1979). Insects, for example, use hydrocarbons for communication between and within species (e.g. species and gender recognition, chemical mimicry, fertility cues, etc.) (Howard & Blomquist, 2005). Prey can use either general or specific predator cues to recognize predator presence. Although using more general cues increases the probability of unnecessary anti-predator behaviour ("over-respond", according to Sih et al. (2010)), it is more likely to recognize invasive predators. While using specific cues is of low costs if prey has evolved efficient escape mechanisms, it increases the chance of not being able to identify invasive predators (Sih et al., 2010).

Besides the lack of avoidance behaviour, body size might be a crucial factor for the invasion success of non-native predators. According to the Metabolic Theory of Ecology (MTE), the maintenance rate of an organism is proportional to its body mass (van der Meer, 2006). Animals with higher body mass have higher metabolic rates and therefore a higher supply rate. Thus, predators with higher body mass would have to consume larger or more prey to cover their maintenance rate and enable growth. Consequently, relatively larger non-native predators are expected to inflict stronger competition. The validity of the MTE has been supported for various arthropod predators (Brose et al., 2008) and it has been suggested that larger non-native predators are stronger invaders than smaller non-native predators (K. Roy et al., 2002;

Schröder et al., 2009), due to body size related advantages (i.e. faster reproduction, stronger competition) (Brockerhoff & Liebhold, 2017; Kajita & Evans, 2010; Ünlü et al., 2020).

Aphids have evolved predator-recognition mechanisms to detect and avoid ladybirds. They use chemical cues and vibration to assess predation risk and to adapt avoidance behaviours (Hatano et al., 2008; Ninkovic et al., 2013; Weisser et al., 1999). Aphid species, such as *Acyrthosiphon pisum* avoid predation by dropping from the host plant, a response that can be costly in terms of a high death rate (Francke et al., 2008; Harrison & Preisser, 2016; Losey & Denno, 1998). Aphids also reduce predation risk by avoiding plants recently occupied by predators (Dill et al., 1990). Hydrocarbons produced by ladybirds, for example for mate choice or as aggregation signals (Hemptinne et al., 1998; Wheeler & Cardé, 2014), can be used by aphids as chemical cues to assess ladybird occurrence and predation risk and to avoid ladybird-rich sites (Ninkovic et al., 2013).

Aphid feeding ladybirds are often used as biological control agents (Hagen, 1962; Hodek, 1967). The multicoloured Asian ladybird *Harmonia axyridis* was introduced to Western Europe in 1982 as a biological control agent in greenhouses, but spread across Europe and became established in many European countries where it was not introduced intentionally (P. M. J. Brown et al., 2007; P. M. J. Brown, Thomas et al., 2011; H. E. Roy et al., 2016; H. E. Roy & Brown, 2015). Due to its wide trophic range and variability in its habitat, *H. axyridis* is a generalist with high competition potential (Adriaens et al., 2003; Kenis et al., 2009; Kenis et al., 2017; H. E. Roy & Wajnberg, 2008). Furthermore, *H. axyridis* is a voracious intraguild predator of *A. bipunctata* and other ladybirds (Burgio et al., 2002; Pell et al., 2008)**;** its arrival and spread has been linked to recent declines in European ladybird populations (Bahlai et al., 2015; P. M. J. Brown, Frost et al., 2011; P. M. J. Brown, Thomas et al., 2011; H. E. Roy & Brown, 2015). Studies have categorized *H. axyridis* as a high risk for native ladybirds, possibly leading to a negative impact, (i.e. through intraguild competition) on the native species' function as biological control agents (Harmon et al., 2007; Kenis et al., 2017; van Lenteren et al., 2008).

In our study, we compared aphid consumption rates of native and invasive ladybirds and assessed aphid avoidance behaviour upon contact with chemical cues of native and invasive ladybirds. For our multispecies-approach, we used the native European ladybirds *Adalia bipunctata*, *Coccinella septempunctata*, *Hippodamia variegata*, *Propylea quatuordecimpunctata* and the invasive Asian ladybird *H. axyridis* as predators. *Acyrthosiphon pisum* were used as the native European prey species (hereafter referred to as aphids). We expected (1) that the larger *H. axyridis* and *C. septempunctata* consume more aphids compared to the smaller ladybird species because of body size related energy demands according to MTE. Furthermore, we expected (2) that *A. pisum* avoids leaves bearing chemical cues of all native ladybird species but not leaves bearing chemical cues of the invasive *H. axyridis*.

Material and Methods

Study organisms

We collected ladybirds from March to September in grasslands and field margins around Marburg, Germany. *C. septempunctata* was collected in 2017, *H. variegata* and *P. quatuordecimpunctata* were collected in 2018, and *H. axyridis* was collected in both years. *A. bipunctata* was purchased from Bioinsecte in Belgium (Adavalue SPRL, Rue Englebert Lescrenier 20, B-4340 Othée), as we could not find sufficient individuals in the wild. The aphids (*A. pisum*) were obtained from the Julius-Kühn Institut (Bundesforschungsinstitut für Kulturpflanzen, Erwin-Baur-Straße 27, 06484 Quedlinburg, Germany). The aphid colony we used was collected at least ten years ago from the field and reared in the laboratory since. The aphids were therefore not confronted with high *H. axyridis* densities in the field and had likely never been in contact with *H. axyridis* prior to the experiment. Aphid behaviour in the experiments therefore reflects their behaviour towards *H. axyridis* at the time of its introduction. For our experiment, the aphids were reared on plants of *Vicia faba* L (Kings Seeds, Monks Farm, Coggeshall Road, Kelvedon, Colchester, Essex, CO5 9PG). Ladybirds, aphids and plants were kept in climate chambers with long-day conditions (16 hours of light, 8 hours of dark), with a constant temperature of 20°C and 65% relative humidity. Ladybirds were kept in small groups, separated by species, in Petri dishes (Ø 9 cm). We provided the Petri dishes with pieces of cellulose paper to offer shelter and increase surface area. The Petri dishes were cleaned and cellulose papers were renewed at least every third day. The ladybirds were fed *ad libitum* with *A. pisum*. Aphids were kept isolated from ladybirds on *V. faba* in plastic containers (10 x 13.5 x 6.5 cm) covered with gauze for aeration. For the experiments, we exclusively used aphids that had no contact to ladybirds prior to the trial.

Predation experiment

The ladybirds were separated in Petri dishes and starved for 24 hours before the start of the experiment. Prior to the start of the experiment, the ladybirds were weighed. We placed 30 aphids in a Petri dish (Ø 9 cm) and added one ladybird individual of the respective species to each Petri dish and repeatedly counted the number of remaining aphids in time intervals of 10, 20, 30, 45, 60, 120, 180, 240, 300, 360 minutes. This trial was replicated with 20-24 different individuals of each ladybird species. Predation experiments comparing *H. axyridis*, *C. septempunctata*, and *A. bipunctata* were conducted in 2017. Experiments with *H. axyridis*, *P. quatuordecimpunctata*, and *H. variegata* were conducted in 2018**.** We obtained a total of 20 replicates of each native ladybird species, with the exception of *A. bipunctata* (24 replicates), and 41 replicates of *H. axyridis* (21 from 2017 and 20 from 2018). Because we found no differences in *H. axyridis* predation rates between the two years (χ^2_1=0.61, P=0.44), we combined the *H.axyridis* replicates from both years for the weight and predation rate comparison between species.

Cue avoidance experiment

In a second experiment, leaves of *V. faba* were prepared twelve hours prior to the experiment as follows. Two connected leaves of one plant were separated and each of them was placed into a separate Petri dish (Ø 3.5 mm). We then placed a ladybird into one of these two Petri dishes to deposit cues on one of the leaves. The second leaf was kept in the same way but without the ladybird, serving as a control. After twelve hours, the ladybird was removed and treatment and control leaves were combined in a new Petri dish (Ø 9 cm). The middle between both leaves was marked. Right after this cue preparation, ten aphids were placed in the middle between both leaves. We then counted the number of aphids on each leaf in time intervals of 10, 20, 30, 40, 50 60, 120, 180 minutes. Cue avoidance experiments using *H. axyridis*, *C. septempunctata*, and *A. bipunctata* as cue donators were conducted in 2017. Experiments with *H. axyridis*, *P. quatuordecimpunctata*, and *H. variegata* were conducted in 2018. We completed a total of 20 replicates per native ladybird species and 40 replicates for *H. axyridis* (20 in 2017 and 20 in 2018). We found no differences in *H. axyridis* cue avoidance between the two years (χ^2_1=1.52, p=0.22). Therefore, we combined the *H. axyridis* replicates from both years for the cue avoidance analysis between species.

Statistical analyses

We used the weight measurements of individual ladybirds recorded prior to the predation experiment to compare body weight between ladybird species. We used a one-way ANOVA, followed by a Tukey post-hoc test for pair-wise species comparison.

To compare aphid consumption (i.e. count data) between ladybird species, we first applied a GLMM with Poisson-error distribution including species identity and observation time as fixed effects and Petri dish and observation identity as random effects to account for repeated measurements and for overdispersion (Bates et al., 2015). For an overall test, we did an ANOVA based on χ^2-statistics (Fox & Weisberg, 2011) followed by a Tukey post-hoc test for pair-wise species comparisons. To test whether aphid consumption of ladybirds is related to body weight, we calculated the same model as above but with body weight (measured from each individual ladybird) instead of ladybird species identity as fixed effect.

To test for ladybird cue avoidance of aphids, we first paired aphid counts on control *versus* treatment leaves (nested per petri dish). Aphids that did not choose one of the two leaves were ignored in the analysis (i.e. the total number of aphids varied between the experimental units – therefore the analysis of proportions). We applied a GLMM with binomial-error distribution including the paired aphid counts and time as fixed effects and Petri dish and observation identity as random effects to account for repeated measurements and for overdispersion. In the first model, we tested for equal distributions of aphids on the two leaves among the ladybird species used for cue deposition (i.e. if aphids actually avoid the cues of the respective ladybird species). In the second model, we tested if aphid leaf-choice differed between the cue donators (i.e. ladybird species identity). For an overall test we calculated an ANOVA (χ^2-statistics, (Fox & Weisberg, 2011)) followed by a Tukey-post-hoc test for pair-wise differences between ladybird species. All statistical analysis were carried out using R , version 3.5.1 (R Core team, 2018).

Results

Body weight differences

Body weight differed significantly between the ladybird species (Fig. 1; $F_{4,120}$=159.2, P<0.001). We found no pairwise differences in body weight between the large ladybird species *H. axyridis* and *C. septempunctata* (z=0.0014, P=0.85). *H. axyridis* and *C. septempunctata* were both significantly heavier than *A. bipunctata*, *H. variegata* and *P. quatuordecimpuncata* (P<0.001).

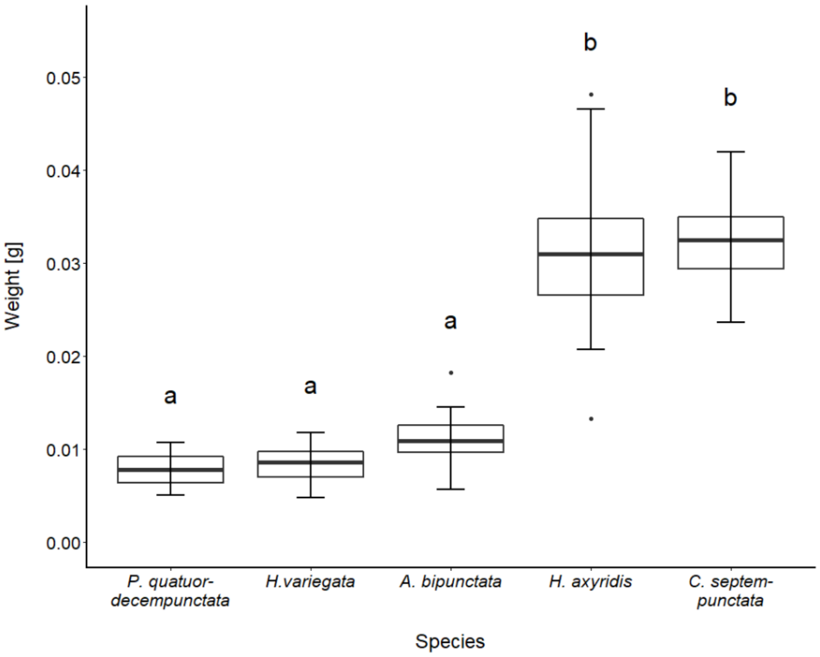

Figure 1. Weight (mean ± SE) of the ladybird individuals per species used in our experiments. Different letters indicate statistically significant differences among the different species accoring to Tukey post-hoc test for pairwise species comparison (P<0.001).

Predation experiment

The number of consumed aphids differed significantly between ladybird species (Fig. 2; χ^2_4=51.48, P<0.001) and increased during the experimental period (χ^2_1=2190.02, P<0.001). The number of consumed aphids did not differ between *C. septempunctata* and *H. axyridis* (z=1.228, P=0.73). However, *H. axyridis* consumed more aphids than *A. bipunctata* (z=4.365, P=<0.001), *H. variegata* (z=5.624, P<0.001) and *P. quatuordecimpunctata* (z=5.257, P<0.001). Similarly, *C. septempunctata* consumed more aphids than *A. bipunctata* (z=2.599, P=0.07), *H. variegata* (z=3.791, P=0.001) and *P. quatuordecimpunctata* (z=3.476, P=0.005). If weight was used instead of species identity in the model, the number of consumed aphids significantly depended on the body weight of ladybirds (χ^2_1=29.089, P<0.001) and time (χ^2_1=2190.301, P<0.001).

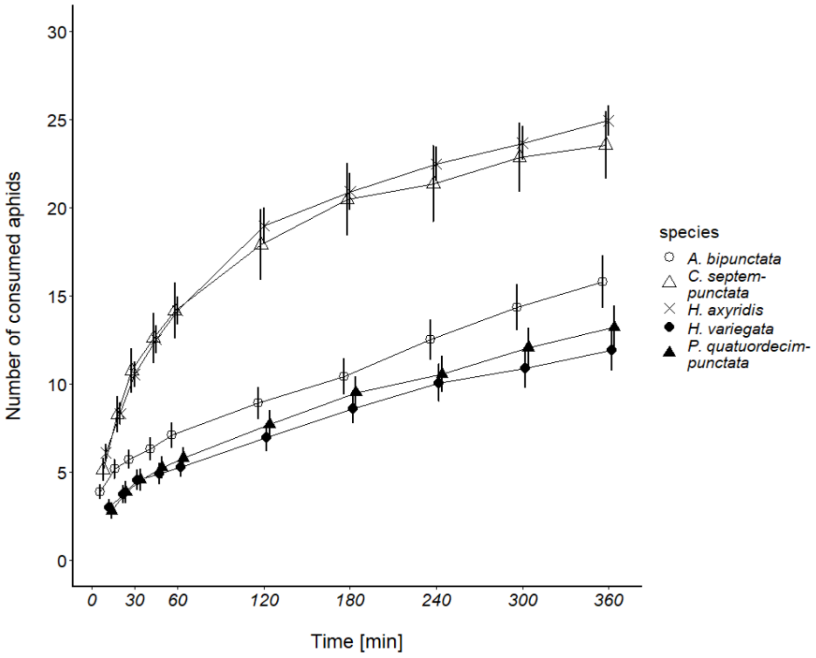

Figure 2. Number (mean ± SE) of consumed aphids over time by the different ladybird species, with an initial aphid density of 30 aphids. Consumed aphids were counted after 10, 20, 30, 45, 60, 120, 180, 240, 300 and 360 minutes. *H. axyridis* consumed more aphids than *A. bipunctata* (P<0.001), *H. variegata* (P<0.001) and *P. quatuordecimpunctata* (P<0.001). *C. septempunctata* consumed more aphids than *A. bipunctata* (P=0.07), *H. variegata* (P=0.001) and *P. quatuordecimpunctata* (P=0.005).

Cue avoidance experiment

Aphids avoided leaves containing cues from *C. septempunctata* (z=-4.006, P<0.001) and *H. variegata* (Fig. 3, z=-2.68, P=0.007). Avoidance of cues from *A. bipunctata* (z=-1.939, P=0.05) was marginally significant. Leaves with *H. axyridis* (z=-0.715, P=0.47) and *P. quatuordecimpunctata* (z=-1.582, P=0.11) cues were not avoided. The proportion of aphids on treatment *versus* control leaves differed between ladybird species (χ^2_4=9.5, P=0.05). Aphid avoidance was stronger in response to cues from *C. septempunctata* compared to cues of *H. axyridis* (z=2.937, P=0.03). We found no differences in the strength of cue avoidance among the remaining ladybird species. There was a marginal significant effect of time on aphid leaf choice (χ^2_1=2.98, P=0.08).

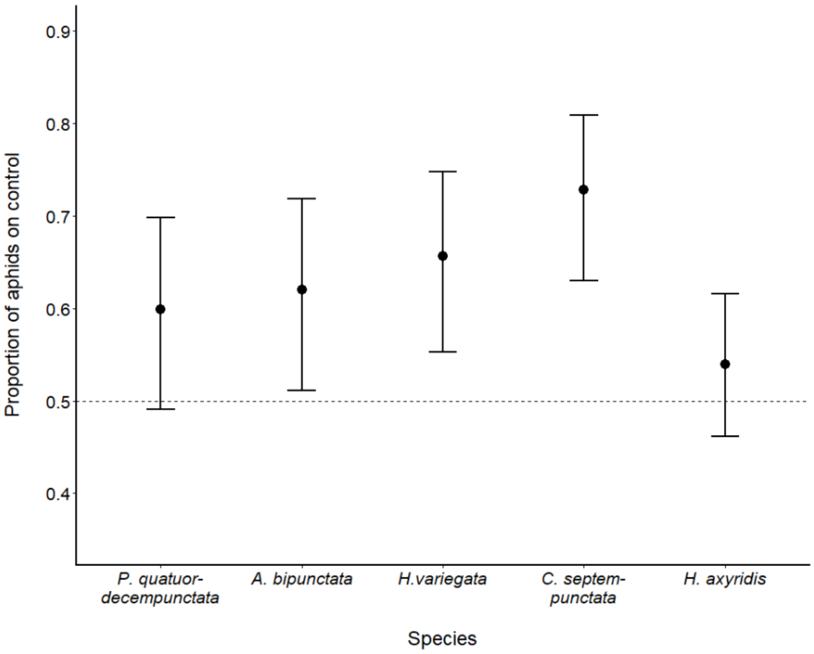

Figure 3. Proportions of aphids on the control leaf without ladybird cues of the respective species (mean ± CI). The dashed line indicates a proportion of 0.5, meaning half of the aphids chose the control (i.e. no ladybird cues) and half the treatment leaf (i.e. containing ladybird cues of the respective species). The proportion of aphids on the control leaf differed significantly from 0.5 for *C. septempunctata* (P<0.001) and *H. variegata* (P=0.007) and marginally significant for *A. bipunctata* (P=0.05). Leaves with cues of *P. quatuordecimpunctata* (0.11) and *H. axyridis* (P=0.47) were not avoided. Aphid avoidance differed between *C. septempunctata* and *H. axyridis* cues and was stronger for *C. septempunctata* (P=0.03).

Discussion

In our study, larger aphidophagous ladybird species consumed more prey than smaller species regardless of the ladybird origin (i.e. native *vs*. invasive). We demonstrated that *A. pisum* is able to detect and avoid chemical ladybird cues. However, avoidance behaviour was only present if confronted with cues of native ladybird species. Cues of the invasive predator *H. axyridis* were not avoided by aphids. These findings suggest naiveté (*sensu* Cox and Lima, 2006) of the pea aphid towards its non-native predator *H. axyridis*.

Our results show that the larger ladybird species, *C. septempunctata* and *H. axyridis,* consume significantly higher numbers of aphids than the three smaller ladybird species. These findings are in line with the MTE, according to which larger animals require a higher food intake to maintain their metabolic rate. There is strong empirical evidence for the body weight-consumption rate relationship as it was shown in spiders and beetles (Brose et al., 2008). We found that the invasive predator *H. axyridis* preys on aphids similarly to *C. septempunctata*, indicating that predation rates are related more to body size than species origin (native *vs.* invasive). We suggest, that aphids rely on general cues (e.g. vibration or alarm pheromones from conspecifics) if directly confronted with ladybirds and can thus detect both native and non-native predator species. In addition, possible differences in predator avoidance upon contact with native and non-native predators (e.g. aphids dropping from plants) are not effective in such simplified predation experiments. However, studies under a more natural experimental setup pointed into the same direction and also showed trends of body size-related dropping and species-related avoidance behaviour of aphids (Ünlü et al., 2020). As possibilities for aphids to escape predation were limited in our experimental setup, actual ladybird consumption rates could differ in the wild. However, our results rather reflected the potential consumption rates of the ladybird species tested. Greater aphid consumption by *H. axyridis* compared to smaller native species can not only have negative impacts on aphid populations and densities, but can also negatively affect smaller native aphidophagous ladybird species that compete with *H. axyridis* for food resources. Besides, larger body size also leads to advantages in life history traits. Reproduction rates, the number of eggs laid per day and the volume of eggs increase with female ladybird size, favouring large ladybirds when prey availability is high (Kajita & Evans, 2010).

We found that *A. pisum* can perceive native ladybird cues and change its behaviour accordingly, a finding that supports previous behavioural experiments demonstrating the perception of chemical cues in various arthropod species (e.g. Bucher et al., 2015; Ninkovic et al., 2013; Oliver et al., 2008). Broad bean leaves which have been exposed to ladybirds were less frequently used for food source by aphids than control leaves. However, only leaves bearing cues of native ladybirds were avoided. Aphids did not avoid cues of the invasive *H.*

axyridis. The aphid colony used in our study was reared in the laboratory for at least ten years and thus had likely never been confronted with high *H. axyridis* densities before. The absence of aphid avoidance behaviour in response to *H. axyridis* cues reflects prey naiveté (according to Cox and Lima (2006)) of *A. pisum* towards its novel predator *H. axyridis* at the time of its introduction. In the field, chemical cues may be used by aphids to avoid settling on plants colonized by ladybirds and thus reduce the risk of predation before any actual encounter. Aphids respond to ladybird cues with increased emigration rate and reduced feeding time, which increases their chance of survival through faster anti-predator response (Tamai & Choh, 2019). Stronger cue avoidance of *C. septempunctata* than *H. axyridis* is of particular interest because these relatively large ladybird species consumed similar numbers of aphids in our predation experiments. Missing avoidance of aphids could have provided *H. axyridis* with a competitive advantage over *C. septempunctata*. Carthey and Banks (2014) suggest that prey detection predation cues weigh the implied risk of predation and the costs of the behavioural reaction against each other. Accordingly, we would expect aphids to avoid cues of the most voracious predators, *C. septempunctata* and *H. axyridis*, more strongly than those of smaller predators. Yet, the opposite was the case for *H. axyridis*. This finding adds information to the mechanism underlying the invasion success of *H. axyridis,* suggesting that it was, in part, facilitated through prey naiveté and increased intraspecific competition potential. However, once aphids are directly confronted with ladybirds, less specific cues like vibration, visual recognition or conspecific alarm pheromones likely outweigh the use of chemical ladybird cues. In this case, it is possible that aphids react to *H. axyridis* in a similar way as they do to native ladybirds. Our findings therefore mainly reflect avoidance reactions prior to any attack.

The negative impact of *H. axyridis* on native ladybird species would depend on the probability of actual encounters in the field. Although *H. axyridis* is present across almost all types of terrestrial habitats, niche overlap is greater with arboreal aphidophagous species like *A. bipunctata* (Adriaens et al., 2007; Kenis et al., 2010). Bahlai et al. (2015) found that the long-term decline of native ladybird species in the field varies with their dietary overlap with the invasive *H. axyridis* and suggested that a decline of *A. bipunctata* since the introduction of *H. axyridis* was most likely due to competitive exploitation. Furthermore, *A. bipunctata* is not only competing for food with *H. axyridis*, but is also particularly susceptible to intraguild predation by *H. axyridis*. Studies already identified declines in *A. bipunctata* following the introduction of *H. axyridis* (P. M. J. Brown et al., 2018; Hautier et al., 2008; Honek et al., 2016). Nevertheless, all native aphidophagous ladybird species can be considered at risk from food competition with *H. axyridis* (Kenis et al., 2010). Prey naiveté could have contributed to exploitative competition between native and invasive ladybird species. Further experiments under more natural conditions would be useful in order to identify how and whether the demonstrated differences in ladybird cue avoidance benefits the invasive *H. axyridis* in the wild. So far, it is likely that prey naiveté, among other factors, facilitated the invasion process of *H. axyridis* in Europe.

H. axyridis is a large invasive ladybird species that consumed higher numbers of aphid prey compared to smaller native ladybird species and thus has the potential to be a strong competitor. Leaves bearing cues of native ladybird species are avoided by *A. pisum*, but not leaves with cues of *H. axyridis*. This demonstrates that *A. pisum* was naïve towards *H. axyridis* at the time of its arrival in Europe and unable to either recognize its cues as dangerous or to adopt appropriate avoidance behaviour. Both relatively large body size and naïve native prey influence predator-prey interactions and likely contributed to the invasion success of *H. axyridis* in Europe.

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CHAPTER 3

Comparison of native and non-native predator consumption rates and prey avoidance behavior in North America and Europe

Ayse Gül Ünlü, John J. Obrycki and Roman Bucher

Abstract

Novel species interactions can contribute to the invasion success of non-native species. Native prey can fail to recognize and avoid non-native predators due to a lack of co-evolutionary history and cue dissimilarities with native predators. This might result in a competitive advantage for non-native predators. Numerous lady beetle species were globally redistributed as biological control agents against aphids, resulting in novel predator-prey interactions. Here, we investigated the strength of avoidance behavior of the pea aphid (*Acyrthosiphon pisum*) towards chemical cues of native lady beetles and non-native Asian *Harmonia axyridis* and European *Coccinella septempunctata* in North America, hypothesizing that cues of non-native lady beetles induce weaker avoidance behavior than cues of co-evolved native lady beetles. Additionally, we compared aphid consumption of lady beetles, examining potential predation advantages of non-native lady beetles. Finally, we compared cue avoidance behavior between North American and European pea aphid populations and aphid consumption of native and non-native lady beetles in North America and Europe. In North America, pea aphids avoided chemical cues of all ladybeetle species tested, regardless of their origin. In contrast to pea aphids in North America, European pea aphids did not avoid cues of the non-native *H. axyridis. Harmonia axyridis* and *C. septempunctata* were among the largest and most voracious lady beetle species tested, on both continents. Consequently, in North America non-native lady beetle species might have a competitive advantage on shared food resources due to their relatively large body size, compared to several native American lady beetle species. In Europe, however, non-native *H. axyridis* might benefit from missing cue avoidance of aphids as well as a large body size. The co-evolutionary time gap between the European and North American invasion of *H. axyridis*, likely explains the intercontinental differences in cue avoidance behavior and might indicate rapid evolution in aphids towards non-native predators.

Keywords: Invasive species; Coccinellidae; Predator-prey interactions; Cue avoidance; Co-evolution

Introduction

Predator-prey interactions shape ecosystems via density- and trait mediated effects (Murdoch et al. 2003, Preisser et al. 2005). Density mediated effects result in the elimination of prey individuals by a predator leading to reduced prey population densities (Murdoch et al. 2003). Prey adapt to the selection pressure of predators by evolving traits that increase the survival during predator attacks (i.e. antipredator behaviors). However, changes in these plastic traits can come at a fitness cost (i.e. non-consumptive effects; Lima and Dill 1990, Peacor and Werner 2000). Fitness costs of trait mediated-effects can reduce prey population densities to a similar extent as density mediated effects (Preisser et al. 2005). Both, density- and trait-mediated effects and can expand into adjacent trophic levels (i.e. trophic cascades or trait-mediated indirect interactions; Terborgh and Estes 2010, Ohgushi et al. 2012).

Predator-induced changes in prey behavior (i.e. antipredator behavior) can enhance prey survival upon predator attacks, interfering with the detection, encounter, and/or capture of prey (Lima 1998). Prey species have sensory mechanisms to detect and recognize cues of co-evolved predators, to effectively respond to a predator attack (Lima and Dill 1990, Rosier and Langkilde 2011). Predator cues serve as sensory information for prey, to recognize co-evolved predators and induce antipredator behaviors. Cues that are involved in interspecific communication can be visual, vibrational cues, and olfactory cues (Fischer et al. 2001, Hermann and Thaler 2014). Chemical cues left by predators persist in nature and can be an indicator of predator presence and future predation risk (Bucher et al. 2014). Lady beetles leave species-specific chemical cues, which are persistent, long-lasting and stick on the surface of plant tissue (Dixon 2000). The species-specific chemical cues left in the tracks of lady beetles consist of cuticular hydrocarbons (Kosaki & Yamaoka 1996) that serve for water proofing (Menzel et al. 2019) and mediate intra- and interspecific communication (Hemptinne et al. 1998, Doumbia et al. 1998, Ninkovic et al. 2013, Menzel et al. 2019). Recently studies revealed that the presence of lady beetle chemical cues on host plants can induce avoidance behavior in psyllids (Seo et al. 2018) and aphids (Ünlü et al. 2020, Ninkovic et al. 2013). The pea aphid *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) consists of numerous distinct biotypes, being adopted to host plants in its local range (Peccoud et al. 2009, Peccoud et al. 2009). Originally of Palearctic origin, North American populations of pea aphids coevolved in agricultural fields with native predators for over a century (Thomas 1878). Missing co-evolution of predator and prey can lead to a lack of detection and recognition mechanisms of predator cues by prey (Cox and Lima 2006). Non-native predators can therefore benefit from a novelty advantage due to lacking or inappropriate antipredator response by prey, leading to higher predation pressure (Sih et al. 2010). Non-native predators can consequently have stronger consumptive effects and weaker non-consumptive effects on prey populations, compared to co-evolved predators. If cues of non-native and native predator species are similar then a similar response can be expected by prey, regardless of predator origin (Sih et al. 2010). Cues of closely related species tend to be more similar, due to similar biosynthetic pathways compared to cues of distantly related species, e.g. chemical cues within lady beetle genera are more similar than between genera (Magro et al. 2010). Thus, relatedness associated with cue similarity between species can lower the impact on prey densities compared to dissimilar non-native predators (Sih et al. 2010).

Lady beetles (Coleoptera: Coccinellidae) have a history of being globally introduced as biological control agents for decades (Harmon et al. 2007). Lady beetles are predators of several pest species (e.g. aphids and coccids), thus providing a valuable ecosystem service in agriculture (Obrycki and Kring 1998, Caltagirone and Doutt 1989). Among the introduced lady beetle species, the European species *Coccinella septempunctata* and *Hippodamia variegata* have been released for biological control of aphids in North America (Angalet et al. 1979, Ellis et al. 1999). The earliest establishment of *C. septempunctata* in North America dates back to 1973 (Angalet and Jacques 1975); The establishment history of *H. variegata* began in 1984 in North America (Gordon 1987). Similarly, the Asian *Harmonia axyridis* was introduced as a biological control agent in North America and Europe (Tedders and Schaefer 1994, Trouve et al. 1997). The introduction of the Asian *H. axyridis* in North America started in 1916 (Gordon 1985), but its earliest establishment was in 1988 (Chapin and Brou 1991). In Europe, *H. axyridis* was introduced in 1995 and the establishment period started in 2000-2001 (Brown et al. 2011). *Coccinella septempunctata* and the Asian *H. axyridis* are relatively large and highly voracious compared to common native aphidophagous species (Elliott et al. 1996, Hoki et al. 2014, Ünlü et al. 2020). Moreover, both species interfere with native trophic interactions associated with a lady beetle species decline in the non-native range, due to resource competition and intraguild predation (Alyokhin and Sewell 2004, Ware et al. 2009), absence of natural enemies (Roy et al. 2011), high abundance (Horn 1991, Koch 2003) and high fecundity (Kajita and Evans 2010) and are therefore classified as invasive species (Roy and Brown 2015). The contribution of cue avoidance behavior of aphids confronted with non-native and native chemical lady beetle cues to the invasion success of non-native lady beetles, remains to be examined.

In this study, we deployed a multi-species approach to compare differences in cue avoidance behavior of a North American population of pea aphid (*Acyrthosiphon pisum*) confronted with chemical cues of the Asian lady beetle species *Harmonia axyridis*, the European lady beetle species *Coccinella septempunctata* and *Hippodamia variegata* and three North American lady beetle species, *Coleomegilla maculata, Coccinella novemnotata* and *Hippodamia convergens.* In addition, we compared aphid consumption rates between all lady beetles tested. We hypothesized (1) missing avoidance behavior of *A. pisum* confronted with cues of the novel non-native *H. axyridis*, lower avoidance behavior confronted with cues of congeneric non-native species (*Coccinella septempunctata and Hippodamia variegata)* compared to cues of native ladybeetle species and stronger avoidance behavior of native lady beetle cues compared to the non-native lady beetle cues tested. (2) We expected higher aphid consumption of the larger non-native lady beetle species *H. axyridis* and *C. septempunctata* compared to smaller lady beetle species, regardless of origin. In addition, cue avoidance and consumption experiments were conducted in Europe, using a European pea aphid population as prey and non-native *H. axyridis*, native *C. septempunctata* and *H. variegata* as predators. We subsequently compared cue avoidance behavior and consumption of North American and European pea aphids confronted with lady beetle species occurring on both continents. We expected (3) weaker avoidance behavior of local aphids towards *C. septempunctata* and *H. variegata* cues in North America compared to Europe (native range) and missing avoidance behavior towards *H. axyridis* cues in North America and Europe. (4) We expected no differences in aphid consumption of *H. axyridis*, *C. septempunctata*, and *H. variegata* between North America and Europe, due to body size related food demands.

Material and Methods

Study species North America

The North American pea aphid *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) colony consists of individuals maintained in a colony started in 1985 at Iowa State University, Ames, Iowa, USA and individuals collected in Lexington, Kentucky in 2003. The colony was maintained in the laboratory (at Iowa State University and the University of Kentucky) on broad bean (*Vicia faba)* plants. They were kept in cages with six to eight pots containing five plants each. Plants were replaced weekly to guarantee a fresh food supply for aphids. Aphids were maintained in the laboratory in climate chambers (22°C ± 1 and a photoperiod of light 16 h: dark 8 h) and in a climatized laboratory (22°C ± 1 and a photoperiod of light 16 h: dark 8 h). The lady beetle species *Coccinella septempunctata and Colleomegilla maculata* were collected in April 2018 in alfalfa fields and in field margins at an agricultural research field station of the University of Kentucky in Lexington, Kentucky, USA. The overwintering generation of *Hippodamia convergens* was obtained from Rincon Vitova Insectaries, Ventura, CA, USA, in April 2018 and stored at low temperatures (5°C). Female and male beetles of these species were subsequently paired in 0.24-liter paper cartons, provided with water and fed *ad libitum* with pea aphids, and frozen *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs (Beneficial Insectary, Redding, CA, USA). Egg clusters laid by individual females were collected and placed into a Petri dish. When larvae hatched, they were separated into glass vials, sealed with cotton, provided with water and fed *ad libitum* with pea aphids and frozen

E. kuehniella eggs until pupation. Individuals of *Harmonia axyrids* were field collected in the pupal stage in May-June and kept in Petri dishes until the adult beetles emerged. *Hippodamia variegata* individuals were collected from an alfalfa field in Le Roy, IL, USA in May/June. *C. novemnotata* was purchased in the larval stage (Lost Ladybug Project, Cornell University, Ithaca, New York 14850), since no individuals were found in Kentucky and separately kept in glass vials (see above) until they developed to adults. The adult lady beetles were subsequently sorted by species and stored in plastic boxes. They were provided with water and fed *ad libitum* with pea aphids, *A. pisum* and frozen *E. kuehniella* eggs and kept at 22 ± 1°C, at a photoperiod of light 16 h: dark 8 h. Voucher specimens were preserved in Ethanol (70%) and stored under -7 ± 1°C at the Department of Entomology (Animal Pathology Building), at the University of Kentucky.

Study species Europe

The European pea aphid colony was obtained from the Julius-Kühn Institut in Braunschweig, Germany, which had been maintained in the laboratory since at least 2007. The aphids were reared on broad bean *V. faba* (v. Sutton Dwarf, Kings Seeds, Manchester) in plastic containers (10.0 x 13.5 x 6.5 cm) covered with gaze for aeration in climate chambers (20 ± 1°C, L:D 16:8 and 65% relative humidity). Aphids were supplied with fresh plants, weekly. The lady beetle species *H. axyridis, C. septempunctata and H. variegata* were collected in June-September in two subsequent years (2017 and 2018) in grasslands around Marburg, Germany. Ladybeetles were kept in small groups, separated by species, in Petri dishes (9.4 × 1.6 cm), fed *ad libitum* with *A. pisum* and were kept at (20 ± 1°C, L:D 16:8 and 65% relative humidity).

Cue avoidance experiments

For the comparison with North American species, beetles were sexed prior to the experiments, to ensure a gender-balanced design (ten male and ten female beetles). Double leaflets of *Vicia faba* were cut in two halves, one control and one treatment leaflet, and placed into Petri dishes (3.5 × 1.0 cm). A single lady beetle was placed on the treatment leaflet in the Petri dish for cue deposition and subsequently removed after 12 hours. The control leaflet remained without a lady beetle. The control and the treatment leaflet were randomly assigned and placed into the center of each half of a Petri dish (9.4 × 1.6 cm). Ten adult aphids were released into the center of each Petri dish. The number of aphids on the control and treatment leaflet were counted after 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0 hours. Twenty replicates were conducted per species in the laboratory under 25.10 ± 0.20 °C and artificial lightning. The leaf-choice experiments in

Europe were identical except that lady beetle individuals were not sexed prior to the experiments, but randomly chosen.

Predation Experiments

Lady beetles were sexed by morphological differences prior to the experiments, accounting for potential intraspecific predation differences of female and male beetles. Beetles were separately placed into small Petri dishes (3.5 × 1.0 cm) and starved for 24 hours prior to experiments. Thirty pea aphids (second to third nymph stage) were counted and placed with a brush into a Petri dish. Beetles were randomly assigned to a Petri dish containing aphids. Aphid predation was quantified by counting the remaining aphids in the Petri dish after six hours. In North America, we freeze-killed (-7 ± 1°C) lady beetle individuals after the experiments and measured body width (widest horizontal distance of closed elytra) and body length (elytral apex to pronotal apex) of all beetles used for the predation experiments under a stereomicroscope. We followed the procedure of Obrycki et al. (1998) to obtain elliptical body area for individual beetles (body area (mm^2) = (π × 0.5 × body length (mm) × 0.5 × body width (mm)). Overall, 20 replicates (ten females, ten males) were conducted per species, in the laboratory under 25.41 ± 0.19 °C and artificial lightning. The predation experiments in Europe were identical, except that lady beetle individuals were randomly chosen and not sexed prior to the experiments.

Statistical analysis

For the cue avoidance experiments in North America and the intercontinental comparison, aphid counts on each leaflet were analyzed as proportions (aphids on control leaf *vs.* treatment leaf). We only considered aphids that made a distinct choice of control or the treatment leaflet. We applied a GLMM with a binomial error distribution to analyze differences between cue donator species identity (i.e. different lady beetle species) on aphid leaf choice. We included cue donator species identity as fixed effects and experimental unit (Petri-dish identity) and an observation level random effect (OLRE) as random effects (to account for repeated measurements and overdispersion). We obtained statistical parameters for the fixed effects via ANOVA (χ2-test) from the R-package car (Fox and Weisberg 2019). Pairwise differences between cue donator species identity were analyzed with a Tukey's contrast test for comparison of means with a Holm correction, to account for familywise error rates, using the glht-function from the multcomp R-package (Hothorn, Bretz & Westfall 2008). We subsequently tested for equal distribution of aphids on control vs. treatment leaf (i.e. if aphids avoid lady beetle cues of the respective cue donator), by applying a GLMM with binomial error distribution. Our fixed effects included species identity and experimental unit (i.e. repeated measurements) and ORLE (accounting for overdispersion) as random effects.

Differences of predation rates in North America after six hours and body area were respectively analyzed with a Games-Howell post-hoc test, following a Welch's ANOVA (F test) accounting for heteroscedasticity. To test the effects of lady beetle species identity, gender and body area on predation rates, we conducted a GLM with lady beetle species identity, gender and body area as fixed effects with a quasi-poisson error distribution. Statistical parameters for the fixed effects were obtained via ANOVA (χ2-test). The intercontinental predation differences between lady beetle species were analyzed with a Games-Howell post-hoc test, following a Welch's ANOVA (F-test). All statistical analyses were performed with the statistical software R, Version 3.4.0 (R Development Core Team, 2017).

Results

Cue avoidance in North America

Aphid leaf choice did not differ between cues of the different lady beetle species (GLMM; $\chi^2 = 5.80$, df = 5, P = 0.33). Aphids avoided plants previously occupied by all species (Fig.1.; *H. axyridis*: $z_{95} = 4.03$, P < 0.01; *C. septempunctata*: $z_{95} = 4.89$, P < 0.01; *C. novemnotata*: $z_{95} = 3.13$, P < 0.01; *Col. maculata*: $z_{95} = 4.63$, P < 0.01; *H. convergens*: $z_{95} = 2.72$, P < 0.01 and *H. variegata*: $z_{95} = 2.35$, P = 0.02; Fig.1).

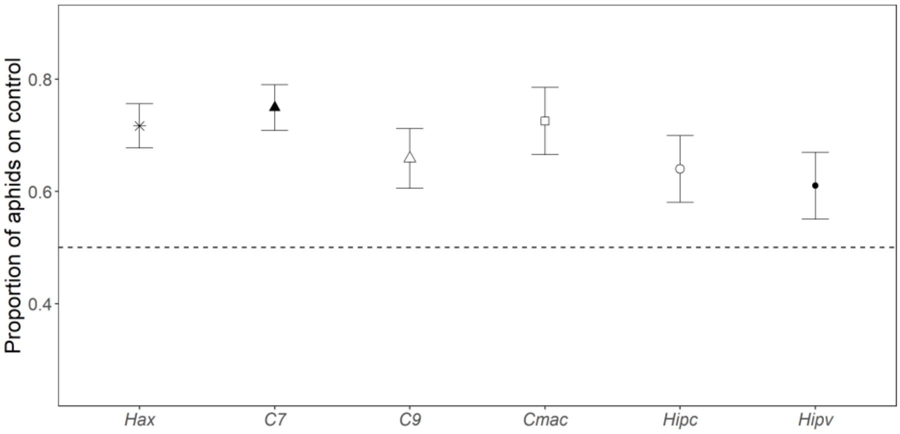

Figure 1. Proportion of pea aphids on cue-free control leaflets (mean ± SE) in North America. Pea aphids avoided leaflets with chemical cues of the native lady beetle species (unfilled symbols) *Coccinella novemnotata* (*C9*), *Coleomegilla maculata* (*C. mac*), *Hippodamia convergens* (*Hipc*) and the non-native lady beetle species (filled symbols) *Harmonia axyridis* (*Hax*), *Coccinella septempunctata* (*C7*) and *Hippodamia variegata* (*Hipv*) (P ≤ 0.02, respectively).

Aphid consumption by lady beetles in North America

The number of aphids consumed after six hours differed among lady beetle species (Welch's ANOVA; F = 26.13, df = 5, P < 0.01). Aphid consumption by *C. septempunctata* and *H. axyridis* did not significantly differ (Games Howell post-hoc test (GH); P = 0.97). There were no differences in aphid consumption between *C. novemnotata* and *H. axyridis* (GH, P = 0.48); Aphid consumption of *C. novemnotata* was lower compared to *C. septempunctata* (GH, P = 0.03). Aphid consumption of *C. septempunctata* and *H. axyridis* was respectively higher compared to *H. convergens*, *Col. maculata* and *H. variegata* (GH; P ≤ 0.02, Fig. 2). Body area differed between lady beetle species (Welch's ANOVA; F = 248.34, df = 5, P < 0.01). There were no body area differences between *C. septempunctata* and *H. axyridis* (P = 0.11) The remaining species (*C. novemnotata*, *H. convergens*, *Col. maculata* and *H. variegata)* were respectively smaller than *C. septempunctata* and *H. axyridis* (GH; P < 0.01). *H. convergens*, *Col. maculata* and *H. variegata were smaller than C. novemnotata*, (GH; P < 0.01). There were no size differences between *H. convergens* and *Col. maculata* (GH; P = 1). *H. variegata* was smaller than *Col. maculata* and *H. convergens* (GH; P < 0.01; Fig. 2). Consumption rates of lady beetles can be explained by differences in species identity (GLM; χ^2 = 23.18, df=5, P< 0.01), beetle gender (GLM; χ^2 = 26.32, df = 5, P < 0.01) and beetle body size (GLM; χ^2 = 6.23, df = 5, P = 0.01).

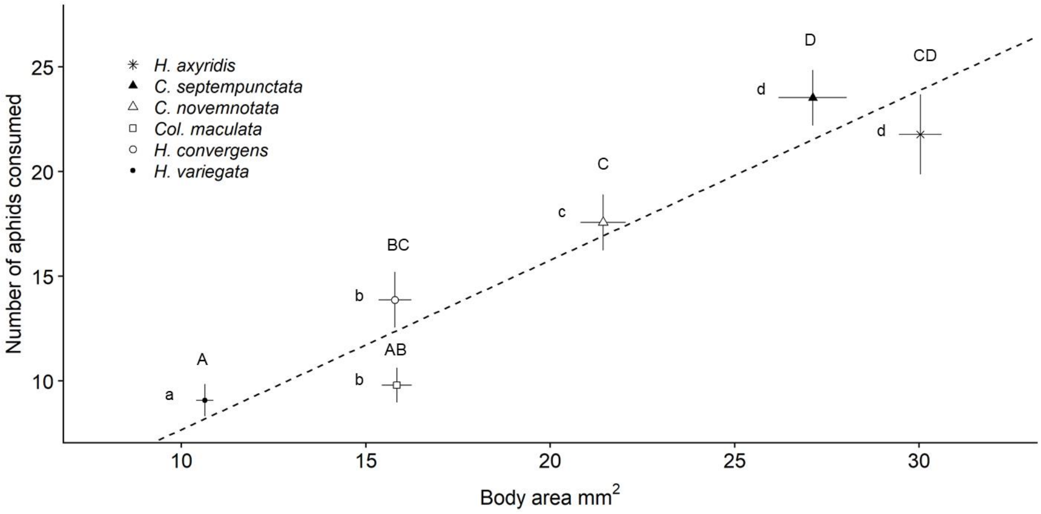

Figure 2. Number of pea aphids consumed after six hours (mean ± SE) and body area (mm^2; mean ± SE) of native (unfilled symbols) and non-native lady beetle species (filled symbols): Different uppercase letters indicate statistical differences of aphid consumption and different lowercase letters indicate statistical differences in body area between species based on a Games-Howell post-hoc test (P < 0.05, same letters do not differ significant). Dotted regression line (y = 0.8133 x - 0.4305; R^2 = 0.54) shows the linear relationship between aphid consumption and body area.

Intercontinental comparison of aphid cue avoidance

Avoidance behavior of local pea aphids differed between cues of different lady beetle species (Glmer; $\chi^2 = 30.56$, df = 5, $P = 0.01$). Avoidance behavior of European aphids was weaker when confronted with *H. axyridis* cues compared to avoidance behavior of North American aphids, *C. septempunctata* cues from both continents and North American *H. variegata cues* (Tukey’s contrasts test (TCT); $P < 0.01$, respectively). Avoidance behavior was marginaly higher when confronted with European *H. variegata* cues compared to European *H. axyridis* cues (TCT; $P = 0.08$). No differences of avoidance behavior were observed between the remaining species (TCT; $P > 0.29$, respectively; Fig. 3).

In Europe, *A. pisum* showed no avoidance confronted with cues of *H. axyridis* ($z_{95} = -1.12$, $P = 0.26$), but avoidance of *C. septempunctata c*ues ($z_{95} = 5.31$, $P < 0.01$) and *H. variegata* cues ($z_{95} = 2.59$, $P < 0.01$). In North America *A. pisum* showed avoidance to *H. axyridis* cues ($z_{95} = 4.429$, $P < 0.01$), *C. septempunctata* cues ($z_{95} = 3.85$, $P < 0.01$) and *H. variegata* cues ($z_{95} = 2.44$, $P < 0.01$; Fig. 3).

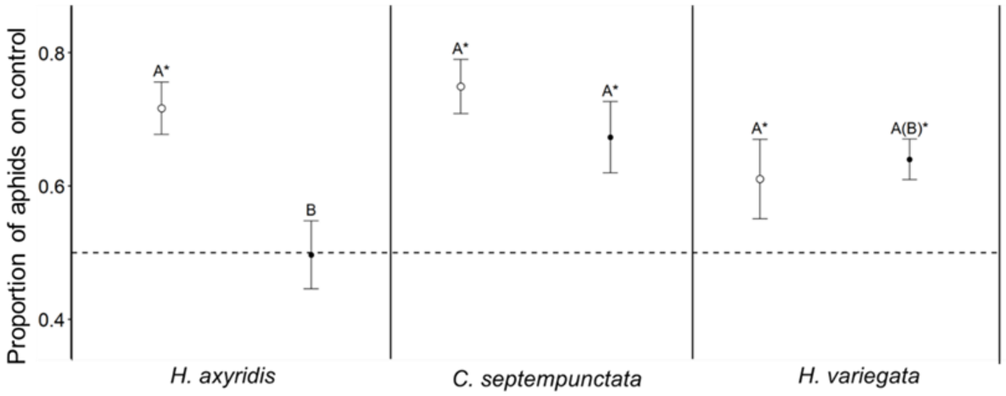

Figure 3. Proportion of aphids on cue-free control leaflets (mean ± SE) in North America (unfilled symbols) and Europe (filled symbols). Asterix indicates significant avoidance behavior of leaflets with chemical cues of lady beetle species ($P < 0.05$). Different letters indicate statistical differences between species based on Tukey’s contrast test ($P < 0.05$).

Intercontinental comparison of lady beetle consumption rates

Predation rates differed among lady beetle species (Welch's ANOVA; F = 42.38, df = 5, $P < 0.01$). North American *H. variegata* consumed fewer aphids than North American and European *C. septempunctata* (GH; $P < 0.1$, respectively) and North American and European *H. axyridis* (GH; $P < 0.1$, respectively). Moreover, *European H. variegata* showed lower consumption rates compared to North American and European *C. septempunctata* (GH; $P < 0.1$, respectively) and North American and European *H. axyridis* (GH; $P < 0.1$, respectively). European and North American aphids were consumed to a similar extent by *H. axyridis* from North America or Europe (GH; $P = 0.52$). Moreover, consumption rates did not differ between European *C. septempunctata* and North American *C. septempunctata* (GH; $P = 1.0$) as well as between European *H. variegata* and non-native North American *H. variegata* (GH; $P = 0.10$). *C. septempunctata* and *H. axyridis* consumption rates did not differ significantly in North America (GH; $P = 0.97$) and in Europe (GH; $P = 0.94$; Fig. 4).

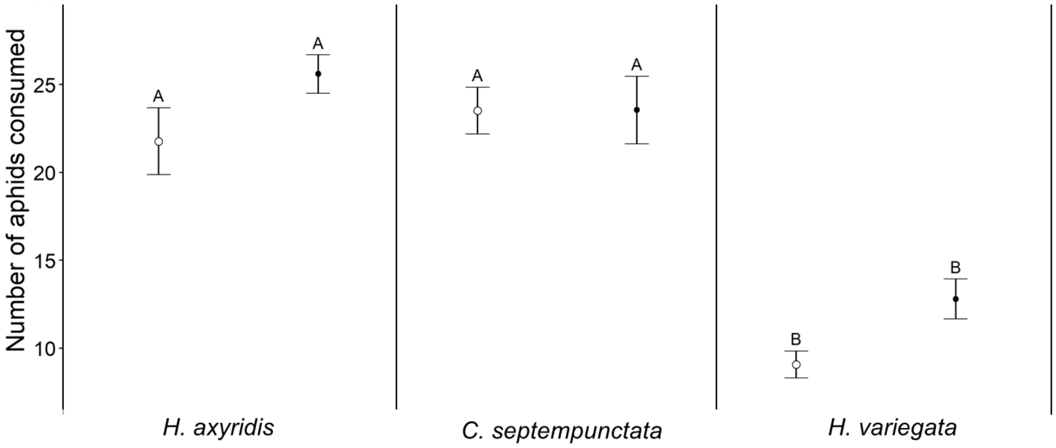

Figure 4. Number of pea aphids consumed after six hours (mean ± SE) of lady beetle species in North America (unfilled symbols) and Europe (filled symbols): Different letters indicate statistical differences of aphid consumption of different lady beetle species based on a Games-Howell post-hoc test ($P < 0.05$, same letters do not differ significantly).

Discussion

In North American pea aphids avoided chemical cues of all lady beetle species tested, regardless of lady beetle origin. In contrast to North American pea aphids, European pea aphids did not avoid cues of the non-native *H. axyridis.* Consumption rates were strongly correlated with body size of lady beetles. On both continents, *C. septempunctata* and *H. axyridis* were the largest species tested and consumed the most aphids.

Contrary to our expectations, North American pea aphids equally avoided chemical cues of all lady beetle species tested, regardless of the origin of lady beetle species. The avoidance of *H. axyridis* cues might be explained by the strong selection pressure by voracious non-native predators on native prey, leading to a rapid evolution of antipredator behaviors (Carthey and Blumstein 2018). In a maritime system, for example, the intertidal snail *Littorina obtusata* responded to the increasing predation pressure of the intertidal crab (*Carcinus maenas*), which was expanding its range, with rapid morphological change of shell forms (Seeley 1986). Moreover, research on cue avoidance behavior in mammals, showed that native common ringtail possum (*Pseudocheirus peregrinus*) recognized and subsequently avoided olfactory cues of the invasive European red fox (Vulpes vulpes), within a few generations of co-evolution (Anson and Dickman 2013). However, in addition to predation risk, the frequency of predator-prey encounters and the length of time since introduction, can be decisive for the evolution of antipredator behaviors (Nelson 2007, Gérard et al. 2014). Prey naïveté decreases with the number of generations since introduction (Anton et al. 2020). The intercontinental comparison of cue avoidance behavior of pea aphids in Europe and North America, indicates only lacking aphid cue avoidance towards European *H. axyridis cues*. Thus, differences of avoidance behavior of pea aphids on both continents could be explained by a difference in co-evolutionary time of non-native *H. axyridis* and pea aphid populations in the field, being shorter in Europe than in North America (Gordon 1985, Brown et al. 2011). In North America, *H. axyridis* was released in multiple agricultural landscapes starting in 1916 (Gordon 1985). Biocontrol agents are repeatedly reintroduced to control agricultural pest species (Lombaert et al. 2014). Thus, our findings suggest that North American pea aphid populations evolved cue avoidance behavior towards *H. axyridis* during the co-evolutionary time spend in shared agricultural fields, which started with *H. axyridis* introduction. In Europe, *H. axyridis* was introduced in the 1990s and the establishment period is estimated to be 2000-2001 (Brown et al. 2011). Here, the co-evolutionary history of *A. pisum* is shorter with *H. axyridis* populations, compared to that of North American populations. Thus, we suggest that differences in the length of co-evolutionary time between *H. axyridis* and pea aphids in North America and Europe can explain the existence of avoidance behavior in North America but not among European pea aphids. Based on our findings, long-term studies could enhance our knowledge on evolutionary changes in non-native predator-prey interactions starting with the initial introduction of non-native predators (Mallon et al. 2015, Anton et al. 2020).

Avoidance behavior of American pea aphids confronted with non-native *C. septempunctata* as well as *H. variegata* cues could be explained by the shared European co-evolutionary history prior to the introduction of pea aphids to North America (Thomas 1878). In the intercontinental comparison, we found that European pea aphids avoided cues of *C. septempunctata* and *H. variegata,* indicating that avoidance behavior is an innate response of European aphids to native *C. septempunctata* and *H. variegata*. Innate antipredator responses to extinct predators can be retained over several generations for decades (reviewed in Parsons et al. 2018); e.g. prey species showed innate avoidance behavior towards olfactory cues of locally extinct predators, even though the extinctions dated 100 years back (Osada, Miyazono & Kashiwayanagi, 2014; Chamaillé-Jammes et al. 2014). According to the 'multipredator hypothesis', prey retains evolved antipredator behaviors towards extinct predators in the presence of remaining predators (Blumstein 2006). Thus, chemical cue avoidance towards non-native European *C. septempunctata* and *H. variegata* might be a retained innate antipredator response of pea aphids, which evolved prior to the introduction of pea aphids to North America in the beginning of the 19th century and remained due to the presence of congeneric native predators (e.g. *C. novemnotata* and *H.convergens).* Moreover, we suggest, that chemical cue similarities between congeneric non-native *C. septempunctata* and *H. variegata* and native *C. novemnotata* and *H.convergens* in North America might contribute to the equally strong avoidance behavior in pea aphids (Sih et al. 2010); Here, cues of non-native and native congeneric lady beetle species can be more similar (Magro et al. 2010) and consequently a similar response can be expected in prey, regardless of predator origin (Sih et al. 2010). However, the degree of cue similarity between the tested lady beetle species remains open and needs further attention. Overall, the 'multipredator hypothesis' in combination with chemical cue similarities of related (congeneric) species might explain cue avoidance of congeneric non-native *C. septempunctata* and *H. variegata* and native *C. novemnotata* and *H. convergens* in North America, as well as the similar strength of avoidance behavior between European and American pea aphids.

In North America and in Europe, the non-native *C. septempunctata* and *H. axyridis* were the largest lady beetles tested (see Ünlü et al. 2020) and consumed the most aphids, compared to smaller native species. Food consumption increases with body mass, due to increasing metabolic requirements (Brose et al. 2008), confirming the positive relationship between lady beetle body area and aphid consumption rates. The successful establishment of a large predator can depend on, i.e. predator size and prey availability (Crookes et al. 2019). In North America, *C. septempunctata* was primarily considered as a non-native biological control agent on pest species, due to large size and voraciousness (Elliot et al. 1996). Predation advantages of invasive *H. axyridis* over the smaller native lady beetle species *Cycloneda sanguinea* on shared pest species, were attributed to a dominance in intraguild interactions, wider dietary range, higher voracity and larger size (Michaud 2002). Moreover, we found, that the smaller sized, non-native *H. variegata* consumes a lower number of aphids, compared to *C. septempunctata* and *H. axyirids* in North America. Thus, asymmetric competition advantages over smaller native and non-native species, can benefit the larger non-native *H. axyridis* on both continents and non-native *C. septempunctata* in North America (Michaud 2002, Hoki et al. 2014). Furthermore, a recent study found that the efficiency of resource utilization was comparatively higher in invasive *H. axyridis* than in native *H. convergens*, when allometric scaling was considered. In addition, aphid handling time was lower and maximum consumption rate was higher in *H. axyridis,* compared to native *H. convergens,* indicating that the invasive *H. axyridis* is the dominating competitor (Crookes et al. 2019). Overall, body size and correlated physiological and/or behavioral traits of invasive species can significantly contribute to a competition advantage towards native and non-native predators (Obrycki et al. 1998, Michaud 2002, Kajita and Evans 2010, Hemptinne et al. 2012).

Non-consumptive effects (i.e. fitness costs) of chemical cue avoidance behavior of pea aphids on aphid populations is likely to comparable between non-native and native lady beetle species in North America. Prior studies showed that disturbances in pea aphid behavior induced by predator cues can lead to increased searching behavior for suitable feeding sides and consequently decreased feeding times, resulting in reproductive costs (Nelson et al. 2004, Nelson 2007). However, predator cues, covering the surrounding area can lead to aphid dispersal to sites with less predator pressure and consequently reduce survival costs through immediate predator consumption (Roitberg et al. 1979). The 'landscape of fear' induces prey to shift to sites with low predation pressure by avoiding sites with high predation risk (Laundre et al. 2001). Another study confirms, that prey can discriminate between risky and suitable feeding sides, by demonstrating that the Colorado potato beetle, *Leptinotarsa decemlineata* reduces feeding on potato leaves covered with predator cues, compared to a cue-free control (Hermann and Thaler 2014). Our findings suggest that chemical lady beetle cues of non-native, as well as native lady beetle species can equally repel aphids and can subsequently serve as proxy for future predation risk. Unexpectedly, non-native lady beetle species do not benefit from missing cue avoidance of pea aphids in North America and fitness costs for pea aphids, inflicted by non-native and native lady beetle species are expected to be comparable. In contrast, pea aphids in Europe, lacking avoidance behavior towards chemical cues of *H. axyridis,* might have lower associated fitness costs, but can suffer higher losses through direct consumption. Our results can further help to explain the current invasion status of *H. axyridis* and European lady beetle species in North America: While the two larger species *H. axyridis* and *C. septempunctata* have spread all over the US within few decades, the smaller

H. variegata, *Propylea quatuordecimpuncata*, and *Adalia bipunctata* kept a more local distribution in the North East (and Noth West for *A. bipuncata*; Gordon 1985, Lost Ladybug Project 2020: www.lostladybug.org). Among other characteristics, large body size can contribute to the invasion success of lady beetles due to increased food competition (see above). Analyzes of a 24-year dataset in southwestern Michigan revealed only significant declines in the relatively small *Col. maculata* and *A. bipunctata* (Bahlai et al. 2015). Likewise, *A. bipuncata* sowed stronger declines in the presence of *H. axyridis* compared to other native lady beetle species, in Europe (Roy et al. 2012). In contrast, *C. novemnotata* maintained an ecological foothold in the face of invasion by the equally sized *C. septempunctata* (Evans 2017). Thus, we suggest that lady beetle body size might be a good predictor for their invasion potential in areas beyond their native ranges. In contrast to body size, differences in predator avoidance can diminish with time. Here, North American aphids but not European aphids avoided cues of the Asian *H. axyridis*. Such rapid evolutionary adaptations can contribute to so called ‘boom-bust dynamics’: invaders go through an initial outbreak before declining to a lower population size (Simberloff and Gibbons 2004, Strayer et al. 2017). So far, evidence for a decline in *H. axyridis* populations is restricted to microsatellite effective population estimates (Sethuraman et al. 2017). In the long term, the evolution of avoidance behavior in native aphids might result in a stable co-existence within the native community and may consequently lead to a more harmless situation relative to the current impact of *H. axyridis*. Rapid evolution in response to non-native predators has important consequences for ecological studies aiming to elucidate the underlying mechanism of biological invasion such as a lack of avoidance behavior: Although lacking avoidance behavior towards non-native predators during early stages of biological invasions benefit the non-native predator, the mechanisms might no longer be detectable at later stages due to rapid co-evolution. Our results in concert with lady beetle distribution data in North America and in Europe indicate that relative lady beetle body size is a key predictor of the invasion success of non-native lady beetle species, but also for native lady beetle species that are at particular risk if they co-occur with non-native lady beetles.

Conclusion

Missing avoidance behavior of European pea aphids towards chemical cues of non-native *H. axyridis*, suggests that non-native predators can benefit from chemical cue novelty resulting in a lack of antipredator behavior of prey, during early stages of biological invasions. In contrast, North American pea aphids showed avoidance behavior towards *H. axyridis* cues, suggesting a rapid evolution of avoidance behavior against voracious, non-native predators. Avoidance behavior towards non-native *H. variegata* and *C. septempunctata* cues might be explained by cue similarities to congeneric native species and co-evolutionary history prior to the introduction of pea aphids to North America. *Harmonia axyridis* and *C. septempunctata* consumed the most aphids compared to native and non-native lady beetle species, due to large body sizes. Overall, predation advantages of non-native predators due to missing antipredator behaviors of prey might diminish with time, whereas body size related competition advantages over smaller native and non-native predators could sustainably benefit large, non-native predators. In addition, long-term studies are required to provide a better understanding of evolutionary changes in non-native predator-prey interactions starting with the initial introduction of non-native predators.

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CHAPTER 4

Predation and avoidance behavior of the pea aphid *Acyrthosiphon pisum* confronted with native and invasive lady beetles in Europe

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Abstract

Invasive species are a major driver of global biodiversity loss. However, we often lack a mechanistic understanding why some non-native species become invasive. Missing co-evolutionary history between invasive predators and native prey can lead to a lack of predator avoidance by native prey and consequently higher consumption rates by invasive predators. We compared predation rates of four native European lady beetle species and the invasive lady beetle species *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), using the native pea aphid *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) for prey. Here, we also quantified initial dropping of *A. pisum* from host plants. In an additional plant-choice experiment, we tested for aphid avoidance of plants bearing lady beetle cues.

Differences in predation were determined by predator body size. Initial dropping of aphids did not differ between the invasive lady beetle species *H. axyridis* and the remaining native lady beetle species. However, *A. pisum* showed no avoidance behavior towards *H. axyridis* cues, but to cues of the most voracious native species. Thus, relatively large body size and missing chemical cue avoidance by aphids can benefit the invasive *H. axyridis.*

Keywords: Predator-prey naïveté, co-evolution, invasion, antipredator response, predator avoidance, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae)

Introduction

Novel species interactions can play a pervasive role in the invasion success of non-native species and their ecological impact (Carthey and Banks 2014). Several evolutionary and ecological hypotheses focus on novel species interactions (Hufbauer and Torchin 2007). The evolution of increased competitive ability (EICA) hypothesis, for example, implies that novel species might benefit from the absence of native top and intraguild predators, reducing the impact on population growth through reduced predation. Thus, the reduced resource allocation to antipredator response in the novel species, leads to a higher competitive ability and consequently to higher fitness (Blossey and Notzold 1995). Besides the absence of predators, missing predator recognition of prey can result in similar consequences for predators: According to the prey-naïveté hypothesis, a lack of co-evolutionary history between native prey and novel predator results in missing species recognition and ineffective antipredator response of native prey, leading to higher consumption rates of the novel predator (Cox and Lima 2006; Sih et al. 2010). While the majority of studies focus on prey naïveté of vertebrates and aquatic organisms (Cox and Lima 2006), there is little scientific understanding of predator-prey-naïveté in arthropods.

Interspecific communication is crucial for species recognition in trophic interactions (Lima and Dill 1990). The assessment of predation risk by prey is mediated by general and/or specific cues (Sih et al. 2010). In arthropods, chemical cues (e.g. cuticular waxes; Dixon 2000; Pasteels 2007) are known to mediate mutualistic and antagonistic interactions (Lang and Menzel 2011; Bucher et al. 2014; Dixon 2000; Mestre et al. 2014). Since predator-specific chemical traces are left by predators on feeding sites, prey species evolved mechanisms to assess and avoid chemical cues of syntopic predators to reduce risk of predation (Ninkovic et al. 2013; Bucher et al. 2015a). Hence, native prey can suffer from heavy predation after the introduction of a non-native species (Sih et al. 2010). However, chemical cue similarity between native and non-native predators could enhance prey recognition and subsequently mediate invasion success (Sih et al. 2010).

Aphids (Hemiptera: Aphididae) have evolved several survival strategies to escape predation, such as kicking, walking away, dropping from the plant and releasing an alarm pheromone to warn neighboring aphids (Villagra et al. 2002; Francke et al. 2008). In addition, aphids can respond to predator presence with the production of winged offspring, allowing for future dispersal (Lees 1966, Weisser et al. 1999). The pea aphid *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) is originally a Palearctic species, which started spreading in the late 19th century and is now globally distributed (Thomas 1878). The species *A. pisum* consists of numerous sympatric populations, characterized by genetic or phenotypic divergence and differences in host plant adaptation in its local range (Peccoud et al. 2009; Peccoud and Simon 2010). Aphids are an essential food resource for several lady beetle species (Coleoptera:

Coccinellidae) (Gordon 1985; Obrycki and Kring 1998), e.g. the Asian multicolored lady beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) is a voracious predator of aphids in agricultural landscapes (Hukusima and Ohwaki 1972). From 1995, *H. axyridis* was introduced as a biocontrol agent in Central Europe (Brown et al. 2008). The period of spread and establishment of *Harmonia axyridis* in most parts of Central Europe ranged from 2002 to 2007 (Brown et al. 2008). Within two decades, this beneficial, non-native species became a successful invader with high dispersal and establishment abilities and wide food spectrum. The spread of *H. axyridis* is associated with the decline of native lady beetles in Europe (Majerus et al. 2006; Van Lenteren et al. 2008; Roy et al. 2012) and North America (Brown and Miller 1998, Alyokhin and Sewell 2004).

We applied a multi-species approach to compare the strength of predator-prey interactions between the invasive Asian lady beetle species *Harmonia axyridis*, four native European lady beetle species, *Coccinella septempunctata* Linnaeus*, Adalia bipunctata* Linnaeus*, Propylea quatuordecimpunctata* Linnaeus*,* and *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae), using the native pea aphid *Acyrthosiphon pisum* for prey. We expected (1) higher aphid predation of *H. axyridis* compared to the native lady beetle species and (2) higher dropping rates if confronted with native lady beetle species compared to *H. axyridis*. In addition, we expected (3) weaker response of *A. pisum* confronted with *H. axyridis* cues, compared to cues of native lady beetle species. This plant choice experiment allowed us to evaluate the role of chemical cues mediating avoidance behavior towards native and invasive lady beetles.

Material and Methods

Study species

Broad bean plants *Vicia faba* Linnaeus (Fabales: Fabaceae) (variety Sutton dwarf; Kings Seeds Essex, UK) were planted weekly in plastic trays (56.5 × 41.5 × 8.5 cm) under room conditions. After seedling emergence, seedlings were potted in groups of six to eight in open plastic containers (18 × 13.5 × 6.5 cm) in a climate chamber (20 ± 1 °C, 65 % relative humidity and 16 h L: 8 h D photoperiod). Pea aphids (*Acyrthosiphon pisum*) were obtained from a laboratory colony of the Julius Kühn-Institut (Braunschweig, Germany), which were maintained in the laboratory for more than ten years. To test for prey naïveté, we chose an unexperienced laboratory aphid population. Here, the aphid laboratory colony was established, when spread and establishment of *H. axyridis* started in Central Europe (see above, Brown et al. 2008). Consequently, this aphid colony has not experienced high *H. axyridis* densities in the field. Aphids were maintained on single (2-3 weeks old) broad bean plants (see above), which were transplanted from plastic containers to plastic cups (11.5 × 12.5 cm) and subsequently covered with gauze for aeration in a climate chamber (20 ± 1 °C, 65 % relative humidity and 16 h L: 8 h D photoperiod). Aphids were transferred weekly to new plants to guarantee fresh food supply. Lady beetles were collected in agricultural and semi-natural habitats from March to August 2017 in Germany, Switzerland and France. Based on observations in field and laboratory (mating activity), the majority of beetles used for the experiments were sexually mature. Individuals of all species, used for the experiments were short-term stored (less than 30 days) under low temperatures (8 ± 1 °C) in plastic collecting tubes (5.3 × 10.0 cm and 3.6 × 8.3 cm) with a water containing microtube for moisture supply, until sufficient numbers of individuals were collected for the respective experiments. Here, cold storage is not expected to have adverse effects on lady beetle behavior in the experiments, due to the short storage period and appropriate storage conditions (Watanabe 2002, Labrie et al. 2008, Ruan et al. 2012). In addition, lady beetles had an acclimatization time of at least 48 hours prior to start of the experiments. Due to an insufficient number of wild *A. bipunctata* individuals found (N = 10), larvae and adults were purchased (Sautter & Stepper GmbH and BioInsecte). At least 48 hours prior to the experiments, lady beetle adults were placed into Petri dishes (9.4 × 1.6 cm) and fed *ad libitum* with pea aphids on single *Vicia faba* leaves. Lady beetles were maintained under constant conditions (20 °C, 65 % relative humidity and 16 h L: 8 h D photoperiod). Plants, lady beetles and aphids were randomly selected for the experiments. All experiments were conducted in microcosms under daylight conditions (natural and supportively artificial lighting) at 24.2 ± 0.8 °C in the laboratory from May-September 2017.

Experimental setup and procedure

Predation and dropping experiments

Lady beetles were starved for 24 hours and singly kept in Petri dishes (3.5 × 1.0 cm). Prior to the experiments, body width (widest horizontal distance between the two closed elytra) of each beetle used for the experiment, was measured under the microscope. Body width is easy to measure on living beetles and used as a proxy for body size (Eberhard 1982). Species-specific body sizes were obtained, to account for size-related metabolic constraints of food consumption (Brose et al. 2008).

Single 2-3-week-old broad bean plants were trimmed to two leaves for standardization and potted into a glass vial (2.2 × 4.5 cm). Fluon® (Polytetrafluoroethylene dispersion) was applied on the stem and the vial, to prevent dropped aphids from climbing back on the plant. Petri dishes (9.4 × 1.6 cm) were treated with Fluon® on the inner walls and subsequently used as ground cover for the experiments. Thirty 2nd-3rd instar aphids were placed on each plant with a brush. Adult aphids were not used in the experiment, due to the possibility of offspring production, increasing the total amount of aphids in our experiments. After an acclimation time of 30 minutes for aphids to settle on the plant, a single lady beetle adult was added at the top of the plant. To prevent lady beetles from escaping, a plastic cup was placed over each plant and petri-dish. Predation was assessed after 0.25, 0.5, 1, 2, 3, 4, 5 and 6 hours, quantifying the remaining number of aphids in each microcosm. Twenty- twenty-three replicates were conducted for each lady beetle species in the predation experiments, divided into ten experimental blocks. Initial dropping was assessed 15 minutes after placing lady beetles on the plants, counting aphids on the ground of each Petri dish. We assessed aphid dropping only at the beginning of the experiment, since dropped and consumed aphids could not be subsequently distinguished. We excluded microcosms from the initial dropping analysis, when beetles were observed to be on the cup or ground after 15 minutes, since aphid consumption by beetles on the ground could have reduced the number of aphids dropped. Thus, the number of replicates differed between the predation and dropping experiments (Predation experiment: *H. axyridis* N = 21, *C. septempunctata* N = 20, *A. bipunctata* N = 20, *P. quatuordecimpunctata* N = 23, and *H. variegata* N = 21; Dropping experiment: *H. axyridis* N = 16, *C. septempunctata* N = 11, *A. bipunctata* N = 17*, P. quatuordecimpunctata* N = 15, and *H. variegata* N = 10).

Cue avoidance experiments

Single standardized broad bean plants (see above) were placed into a petri dish, the plant roots were subsequently covered with soil and the ground was than leveled and compacted. Three lady beetles of the same species were placed on single treatment plants. During 24 hours, lady beetles were allowed to walk on the broad bean plants to deposit their chemical cues (e.g. cuticular hydrocarbons). Control and treatment plants were subsequently covered with perforated plastic cups (9.5 × 10.5 cm).

After 24 hours lady beetles were removed from the treatment plants. A single treatment and a control plant were positioned opposite each other in a terrarium (23.0 × 15.3 × 16.5 cm) and the remaining bare ground around the petri-dishes was covered with soil (see Bucher et al. 2015b). Prior to the start of the experiments 30 aphids (adult and fourth instars) were placed with a brush into a small Petri dish (3.5 x 1.0 cm). Aphids were subsequently released in the center of the terrarium on neutral ground. Terraria were covered with perforated cling film to prevent aphids from leaving. The number of aphids on treatment and control plant were quantified after 0.5, 1, 1.5, 2, 3, 4 and 6 hours. The position of treatments in terraria and cups were randomized prior to the experiments, to account for potential position-related confounding factors. Twenty replicates per lady beetle species were conducted, divided into ten experimental blocks.

Statistical analysis

To test the effect of lady beetle species on aphid predation over time, we used a Generalized Linear Mixed Model (GLMM, lme4 package, Bates et al. 2015) with a poisson error distribution. The fixed effects included species and time and the random effects experimental unit (i.e. repeated measurements) and Observation-Level Random Effect (OLRE), accounting for overdispersion, were added to the model. We obtained statistical parameters for the fixed effects via ANOVA (χ^2 test) from the R package 'car' (Fox and Weisberg 2019). To test for differences between aphid predation of lady beetle species we used a Tukey's contrast test for comparison of means with a Bonferroni correction to account for familywise error rates, by using the glht function from the multcomp package (Hothorn, Bretz & Westfall 2008). Differences of predation rate after six hours (last time point) and body width were respectively analyzed with a Games-Howell post-hoc test, following a Welch's ANOVA (F test) accounting for variance inhomogeneity.

To test the effect of beetle body width on predation after six hours (last time point), we used a Generalized Linear Model (GLM) with a quasi-poisson distribution (accounting for overdispersion). Body width and species were included as fixed effects in the model. Statistical parameters for the fixed effects were obtained via ANOVA (χ^2 test).

In the dropping experiment we tested the effect of lady beetle species on aphids dropped, using a GLM with a quasi-binomial error distribution (accounting for overdispersion). Aphid counts entered the model as proportions (aphids on plant *vs.* dropped) and species was included as fixed effect. We obtained statistical parameters for the effect of species via ANOVA (χ^2 test). Multiple comparisons of means were achieved by using Tukey's contrasts method, with a Bonferroni correction.

In the cue avoidance experiment, aphid counts were analyzed as proportions (aphids on control plant *vs.* treatment plant). We only considered aphids that made a distinct choice of the control or the treatment plant. Therefore, aphids on the ground and terrarium wall were excluded from the analysis. We used a GLMM with a binomial distribution to see the effects of species and time on aphid plant choice. We included species and time as fixed effects and experimental unit and OLRE (accounting for overdispersion) entered the model as random effects. We obtained statistical parameters for the fixed effects via ANOVA (χ^2 test). We subsequently tested for equality of proportions of aphids on control *vs.* treatment plants, by applying a GLMM with binomial error distribution. Our fixed effects included species and as random effects we added experimental unit (i.e. repeated measurements) and OLRE (accounting for overdispersion) to the model.

Statistical analyses were performed with the statistical software R, Version 3.4.0 (R Development Core Team, 2017).

Results

Predation and dropping experiment

Species significantly affected predation rate over time (GLMM, $\chi^2 = 23.33$, *df* = 4, P < 0.01). The number of aphids consumed differed between ladybeetle species; *H. axyridis* and *C. septempunctata* consumed higher numbers of *A. pisum* than *H. variegata* and *P. quatuordecimpunctata* over a six-hour feeding period (Tukey's contrast test *P* < 0.02, respectively; Fig. 1). Predation rates of *A. bipunctata* did not differ significantly from *H. axyridis* and *C. septempunctata* (Tukey's contrast test P = 0.17 and 0.10, respectively; Fig. 1). No differences in predation rates were observed between *C. septempunctata* and *H. axyridis* (Tukey's contrast test P = 1.0; Fig. 1) as well as between *H. variegata*, *A. bipunctata* and *P. quatuordecimpunctata* (Tukey's contrast test P > 0.8, respectively; Fig. 1).

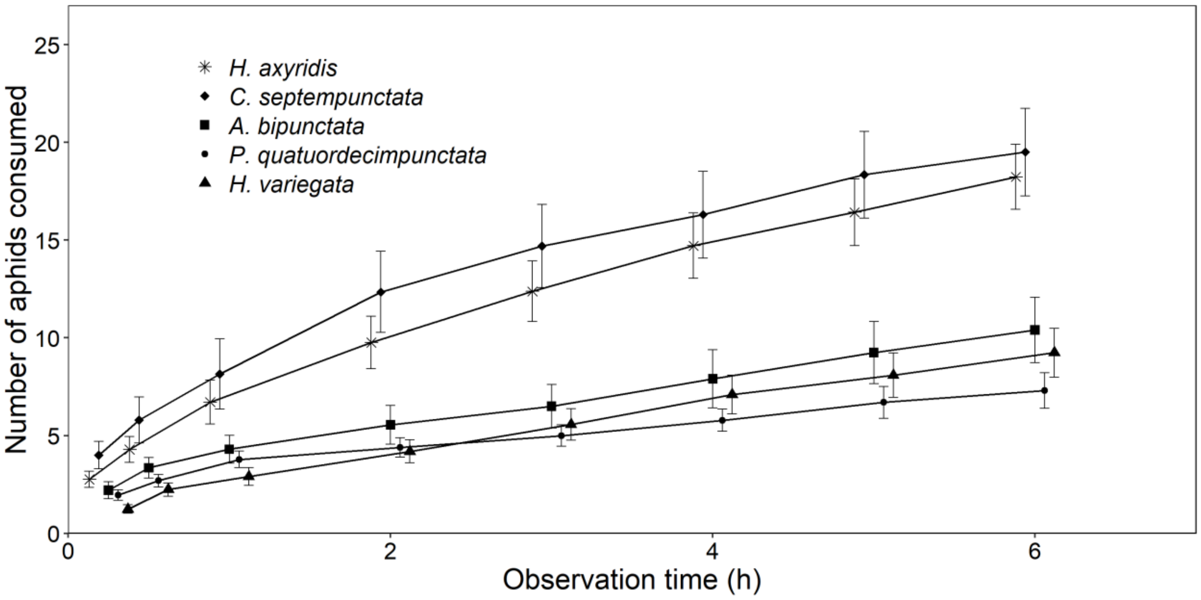

Figure 1. Number of aphids consumed (mean ± SE) by the invasive *Harmonia axyridis* and native lady beetle species (*Coccinella septempunctata, Adalia bipunctata, Propylea quatuordecimpunctata,* and *Hippodamia variegata*) over a six-hour feeding period.

If only the last time point of six hours was analyzed, predation rates differed between species (Welch's Anova, $F_{4,47.85} = 12.41$, $P < 0.01$). *C. septempunctata* and *H. axyridis respectively* consumed more aphids than *A. bipunctata, H. variegata* or *P. quatuordecimpunctata* after 6 hours (Games-Howell test, $P < 0.02$, respectively; Fig. 2). Predation rates between *C. septempunctata* and *H. axyridis* did not differ (Games-Howell test, $P > 0.99$; Fig. 2). No differences could be observed between *H. variegata*, *A. bipunctata* and *P. quatuordecimpunctata* (Games-Howell test, $P > 0.50$, respectively; Fig. 2).

Body size of species differed (Welch's Anova, $F_{4,48.63} = 374.67$, $P < 0.01$). *H. axyridis* and *C. septempunctata* were the largest species used in our experiments (Games-Howell test, $P = 0.53$; Fig. 2), compared to the smaller *A. bipunctata*, *P. quatuordecimpunctata* and *H. variegata* (Games-Howell test, $P < 0.01$, respectively; Fig. 2). *A. bipunctata* was larger than *P. quatuordecimpunctata* and *H. variegata* (Games-Howell test, $P < 0.02$ respectively; Fig. 2). The predation rate of beetles can be explained by differences in beetle body size (GLM, $\chi^2 = 5.68$, *df* = 1, $P < 0.02$) at the last time point of six hours. If body size was included in the model, aphid predation rates were no longer explained by lady beetle species identity (GLM, $\chi^2 = 4.76$, *df* = 4, $P = 0.31$).

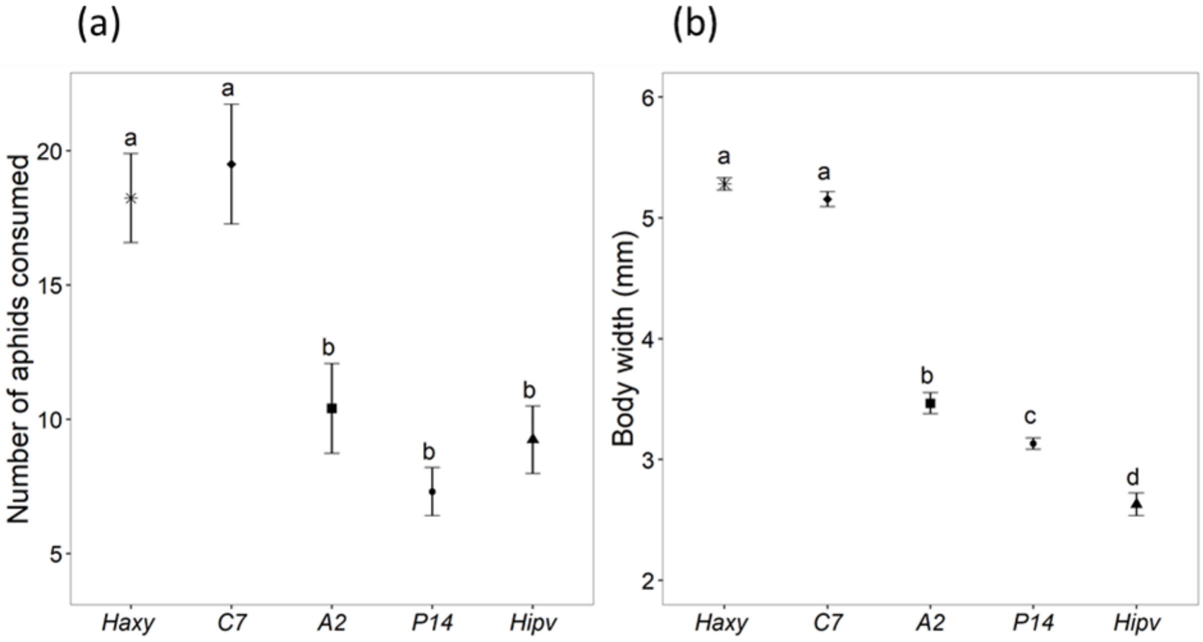

Figure 2. Number of aphids consumed (mean ± SE) by the invasive *Harmonia axyridis* and native lady beetle species (*Coccinella septempunctata, Adalia bipunctata, Propylea quatuordecimpunctata,* and *Hippodamia variegata*) after six hours (a). Body width (mean ± SE) in mm of lady beetle species used for the predation and dropping experiments (b). Different lower-case letters indicate statistical differences based on a Games-Howell post-hoc test ($P < 0.05$).

Initial dropping rate of *A. pisum* varied among ladybeetle species (GLM, $\chi^2 = 16.16$, *df* = 4, $P < 0.01$). Significantly lower numbers of aphids dropped after encounters with *P. quatuordecimpunctata* compared to *C. septempunctata* (Tukey's contrast test $P < 0.01$; Fig. 3). Moreover, marginally lower numbers of aphids dropped after encounters with *A. bipunctata* compared to *C. septempunctata* (Tukey's contrast test $P < 0.07$; Fig. 3). We observed no further significant differences in aphid dropping rate between the different lady beetle species ($P > 0.2$, respectively; Fig. 3).

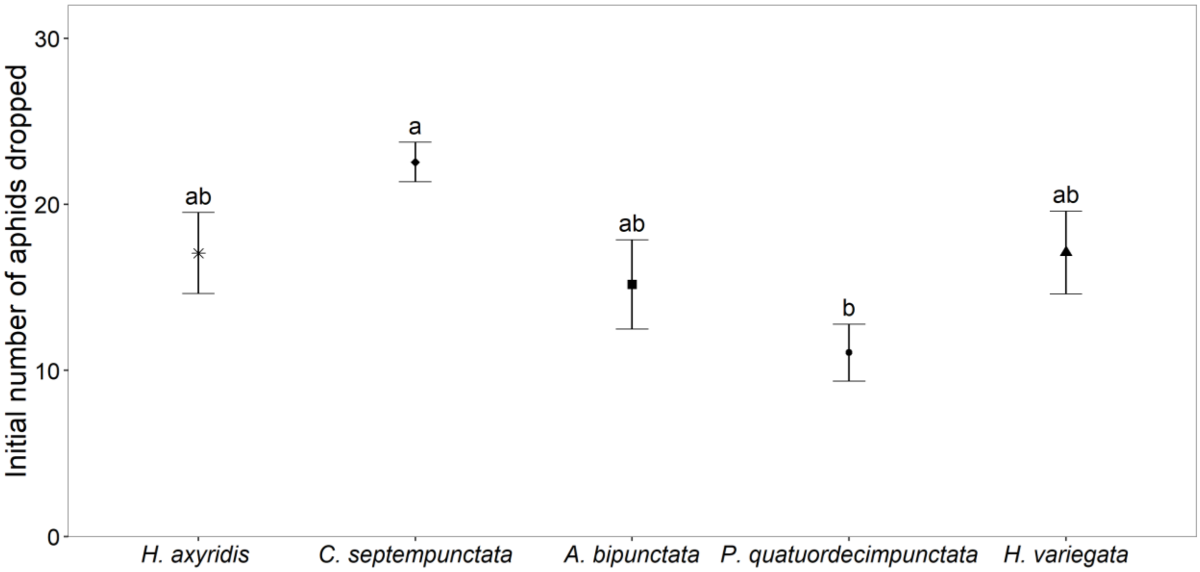

Figure 3. Number of aphids dropped (mean ± SE) in the presence of a single lady beetle after 15 minutes. Differences in dropping rate of lady beetle species were analyzed by Tukey's contrast test for comparison of means ($P < 0.05$, same letters do not differ significantly).

Cue avoidance experiment

Aphid plant choice was marginally affected by lady beetle species (GLMM, $\chi^2 = 8.18$, $df = 4$, $P = 0.09$) and the effect of time was not significant (GLMM, $\chi^2 = 0.65$, $df = 1$, $P = 0.42$). The test for equality of proportions on control plant *vs.* treatment plant showed, that aphids avoided plants previously occupied by *C. septempunctata* ($z_{95} = 2.00$, $P < 0.05$) (Fig. 4) and showed preference towards the control plant. We observed no avoidance behavior towards cues of *H.axyridis* ($z_{95} = -0.24$, $P = 0.81$), *A. bipunctata* ($z_{95} = -0.99$, $P = 0.32$), *P. quatuordecimpunctata* ($z_{95} = 1.08$, $P = 0.28$) and *H. variegata* ($z_{95} = -1.43$, $P = 0.15$) (Fig. 4).

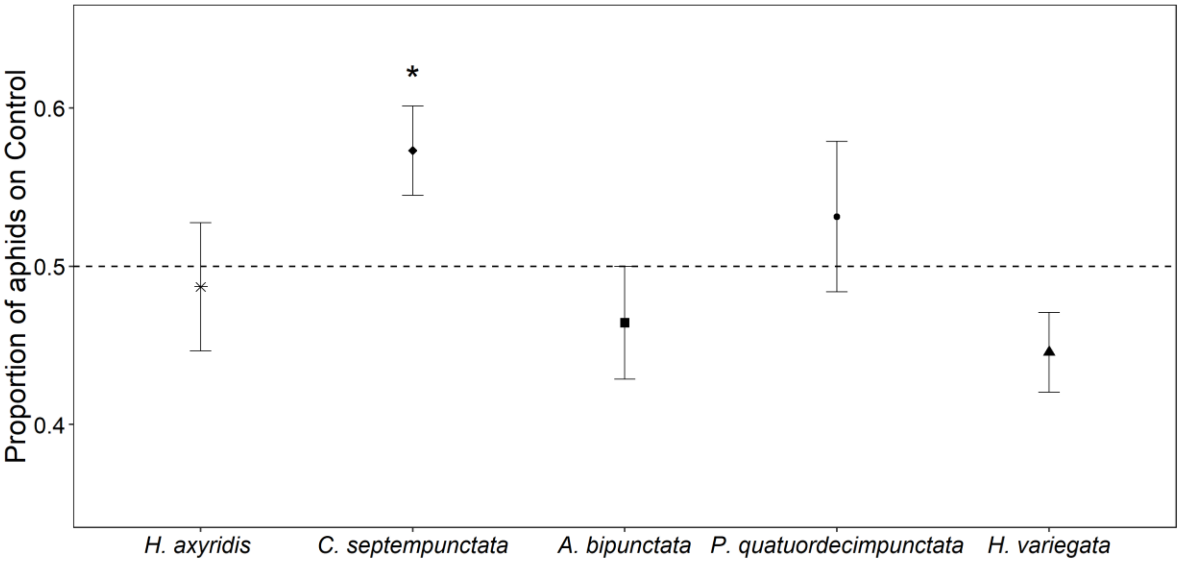

Figure 4. Proportion of aphids on control plant (mean ± SE) compared to treatment plant with cues of different lady beetle species. GLMM results indicate avoidance against *C. septempunctata* cues ($z_{95} = 2.0$, $P < 0.05$) but not against cues of *H. axyridis* ($z_{95} = -0.24$, $P = 0.81$), *A. bipunctata* ($z_{95} = -0.99$, $P = 0.32$), *P. quatuordecimpunctata* ($z_{95} = 1.08$, $P = 0.28$) and *H. variegata* ($z_{95} = -1.43$, $P = 0.15$).

Discussion

Aphid predation rates of *H. axyridis* and*. C. septempunctata* did not differ significantly. *A. bipunctata, H. variegata* and *P. quatuordecimpunctata* respectively consumed significantly fewer aphids than *H. axyridis* or *C. septempunctata* after six hours. There were no significant differences in predation rates between *H. variegata*, *P. quatuordecimpunctata* and *A. bipunctata*. Here, differences in predation rates can be better explained by beetle body size than by species identity. *C. septempunctata* caused significantly higher dropping rates than *P. quatuordecimpunctata*. Unexpectedly, no differences in aphid dropping could be seen between *H. axyridis* and the remaining species. Predator avoidance was only observed in treatment plants bearing *C. septempunctata* cues, compared to the untreated control plant. *A. pisum* did not avoid cues of *H. axyridis* and the remaining native species.

Differences in predation among the lady beetles used in our experiments can be explained by beetle body size, being comparable in *C. septempunctata* and *H. axyrids,* but lower in the remaining native species. No significant differences in predation rates over the whole observation period between *A. bipunctata* and *H. axyridis* or *C. septempunctata* can be explained by smaller interspecific predation differences at the earlier time points, which increased with time (see Fig. 1). Moreover, intraspecific body size differences (i.e. a few considerably larger individuals) between larger wild individuals and smaller laboratory individuals resulted in higher intraspecific variation *of A. bipunctata* predation rates compared to the other lady beetle species tested. The consumption increases with body size, due to developmental requirements (Hodek 1973). Thus, rather than advantages in predation due to a lack of co-evolutionary history, beetle size considerably influences aphid predation. Our results are in line with previous body size related quantitative predation studies of lady beetle species (Finlayson et al. 2010; Mishra et al. 2011). Beetle size and predator efficiency of non-native lady beetle species on shared food resources (Obrycki et al. 1998; Majerus et al. 2006; Roy et al. 2012; Hoki et al. 2014) can be factors contributing to the decline of native lady beetle species.

In the predation experiments, aphids were confronted with physically present predators and therefore immediate predation risk, inducing aphid dropping. Dropping behavior is an antipredator response to escape larger predators, like aphidophagous lady beetles, which pose a high predation risk (Evans 1976; Losey and Denno 1998). Contrary to our expectations, aphid dropping rates induced by the presence of the invasive *H. axyridis* are comparable to those of native lady beetle species. Previous studies on coccinellid induced aphid dropping, observed an increase in dropping behavior with increasing body size of the predators (Hoki et al. 2014; Francke et al. 2008; Evans 1976; Losey and Denno 1998). Increasing size ratios between predator and prey can lead to the evolution of stronger antipredator response (Evans 1976; Binz et al. 2014). *H. axyridis and C. septempunctata* are relatively large, however smaller native species elicited similar dropping rates in pea aphids, indicating that further cues or species traits could induce aphid dropping. Here, a relatively large, moving object can be considered as a general predator cue (Dill 1974; Sih 1986; Sih et al. 2010), initiating aphid dropping. In addition, higher activity of a predator can result in higher dropping rates of *A. pisum,* due to increased vibration or more potential aphid encounters (Francke et al. 2008). Furthermore, aphids attacked by a predator, can release an alarm pheromone (Bowers et al. 1972; Kislow and Edwards 1972; Nault et al. 1973), which can lead to dropping behavior of surrounding aphids (Roitberg and Myers 1978; Dill et al. 1990). In summary, we suggest that cues eliciting dropping behavior in pea aphids are general predator cues, which *H. axyridis* and native species have in common.

While dropping is a response to immediate predation risk, avoidance behavior towards chemical cues left by predators are a proxy for future predation risk. Aphids can leave the current host plant and move to neighboring host plants, due to e.g. predator disturbance (Roitberg et al. 1979) or water stress (Honěk et al. 1998). Since younger instars are limited in movement capacities, adults and older instars preferably walk away and look for neighboring host plants (Roitberg et al. 1979, Honěk et al. 1998). Consequently, adult and forth instar aphids, used in the cue avoidance experiments were able to choose between plants and were not impaired in movement activity, due to the developmental stage. We expected that aphids are more likely to respond to chemical cues of coevolved native predators and show a lack of recognition towards the chemical cues of the invasive predator. Aphids showed no avoidance to *H. axyridis* and three native lady beetle species (*A. bipunctata, H. variegata* and *P. quatuordecimpunctata*), but against *C. septempunctata* cues. This finding is consistent with the results of Ninkovic et al. (2013), who showed that *C. septempunctata* cues repelled cherry oat aphids *Rhopalosiphum padi* Linnaeus (Hemiptera: Aphididae) from barley plants *Hordeum vulgare* Linnaeus (Poales: Poaceae). Thus, aphids can detect chemical traces of coccinellid predators and subsequently avoid feeding sites (Ninkovic et al. 2013). Predation risk can further induce the production of winged aphid offspring, accounting for the survival of the next generation on new plants (Dixon and Agarwala 1999). Chemical cues of lady beetles consist of numerous chemical compounds (Hemptinne et al. 1998, Hemptinne and Dixon 2000). Although the composition of chemical cues are species specific, certain compounds can appear across species (Magro et al. 2010). Native and non-native lady beetle species, bearing similar cues, could induce comparable prey avoidance. Thus, cue similarity between species should be considered, when testing for cue recognition. Magro et al. (2010) found that chemical cue composition of invasive *H. axyridis larvae* differed significantly from those of native *A. bipunctata* and *C. septempunctata* cues, whereas cue compositions of the two native species showed higher similarities. Since avoidance was only observed in treatment plants bearing *C. septempunctata* cues, our results indicate that species specific cues are involved (Sih et al.

2010). However, the chemical compounds in adult *C. septempunctata* cues that induce aphid avoidance are not identified and it remains unknown, if they are part of the chemical profile of the other lady beetle species. Contrary to our hypothesis, *A. pisum* did not avoid cues of the smaller native species. We suggest that larger predators exert stronger selection pressure on prey (see Binz et al. 2014). Antipredator responses are associated with costs and therefore depend on the degree of risk perceived by the prey (Kats and Dill 1998; Carthey and Banks 2014). According to our results, *C. septempunctata* is the most voracious predator among the tested native species, possibly inducing cue avoidance as an evolutionary response of *A. pisum* to avoid severe future predation risk. Our results further indicate that, cues of the equally voracious *H. axyridis* did not repell *A. pisum*, suggesting that *A. pisum* could not evolve adequate predator recognition, due to a shorter co-evolutionary time with *H. axyridis.*

Conclusion

We suggest, that *H. axyridis* has a competition advantage when preying on pea aphids compared to smaller native species, due to predator size rather than due to missing predator recognition. Immediate risk by the presence of a predator elicits dropping behavior in *A. pisum*, regardless of the origin of the lady beetle species. However, *A. pisum* responds only to chemical cues of the native *C. septempunctata*. A higher selection pressure of larger predators on *A. pisum*, might explain the missing cue avoidance towards the three smaller native species, whereas predator-naïveté might be the cause for missing chemical cue recognition of the large, invasive *H. axyridis*. Although *H. axyridis* and *C. septempunctata* consumed similar numbers of aphids in our laboratory experiment, lower aphid predation rate of *C. septempunctata* might be expected under natural conditions, due to stronger cue avoidance of *A. pisum*. Our results indicate that size differences with native predators as well as missing cue avoidance can contribute to the invasion potential of non-native species.

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CHAPTER 5

Interactions of ants with native and invasive ladybeetles and the role of chemical cues in intraguild interference

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Abstract

The predator-predator naïveté hypothesis suggests that non-native predators benefit from being unknown to native predators, resulting in reduced intraguild interference with native predators. This novelty advantage should depend on the ability of native predators to recognize cues of non-native predators. Here, we compared ant aggression and ladybeetle reaction in four native and the invasive ladybeetle species *Harmonia axyridis*. In addition, we tested whether ladybeetle cuticular hydrocarbons (CHCs) are involved in species recognition, which might explain naïveté if the invasive species has a specific CHC profile. To this end, we conducted behavioral assays confronting two native ant species with both living ladybeetles and ladybeetle elytra bearing or lacking CHCs of different ladybeetle species. Finally, we characterized CHC profiles of the ladybeetles using GC-MS. In general, aggression of *Lasius niger* was stronger than that of *Myrmica rubra*. Both, *L. niger* aggression and ladybeetle reaction were stronger in *Coccinella septempunctata* compared to the invasive *H. axyridis*. The removal of CHCs from ladybeetle elytra reduced aggression of both ant species. If CHCs of respective ladybeetle species were added on cue-free elytra, natural strength of *L. niger* aggression could be restored. CHC analyses revealed a distinct cue composition for each ladybeetle species. Our experiments demonstrate that the presence of chemical cues on the surface of ladybeetles contribute to the strength of ant aggression against ladybeetles. Reduced aggression of *L. niger* towards *H. axyridis* compared to the equally voracious *C. septempunctata* might improve the invasive ladybeetle's access to ant-tended aphids.

Keywords: biological invasion, species interaction, ant aggression, cuticular hydrocarbons, Coccinellidae, Formicidae

Introduction

Non-native species invading areas beyond their native ranges are often a major threat to biodiversity (Lodge 1993; Mack et al. 2000; Bax et al. 2003) and their numbers are likely to increase due to worldwide traveling and transportation of goods (Lodge 1993; Hulme et al. 2008). Detrimental effects of invasive species on native species are well documented in the scientific literature, but we often lack a mechanistic understanding of the invasion success (Hayes and Barry 2008; Blackburn et al. 2011). A number of ecological and evolutionary hypotheses have been proposed aiming to explain the invasion success of non-native species (see Hufbauer and Torchin 2007), many of which focus on the role of biotic interactions. The 'enemy release hypothesis', for example, proposes that non-native species can benefit from missing predators (Keane and Crawley 2002; Colautti et al. 2004). Nevertheless, even if predators are present, the lack of shared evolutionary history between native prey and a non-native predator can facilitate the establishment of non-native predators because of lacking predator recognition, also known as 'predator-prey naïveté hypothesis' (Cox and Lima 2006; Carthey and Banks 2014).

Predation risk is one of the great driving forces of prey populations (Lima and Dill 1990) and invasive predators, in particular, are causing rapid extinctions or declines in many native prey species (Mooney and Cleland 2001; Carthey and Banks 2014). Besides predator-prey systems, predator-predator interactions can contribute to invasion success as well (Finlayson et al. 2009). Killing of other predators (i.e. intraguild predation) or interference with other predators (i.e. intraguild interference) is common in food webs and can hamper top-down control of predators (Polis et al. 1989; Arim and Marquet, 2004). Studies on the invasion success of non-native predators should thus consider intraguild interactions (Vance-Chalcraft and Soluk 2005). So far, the empirical evaluation of hypotheses aimed to explain invasion success is biased towards plants, vertebrates and aquatic organisms while terrestrial insects received relatively less attention (Parker et al. 1999; Bax et al. 2003) despite the fact that they represent a large part of the alien fauna (Kenis et al. 2009).

In insects, chemical cues play an important role for intra- and interspecific communication (Howard and Blomquist 2005; Monnin 2006). Chemical recognition is best known in social insects, where cuticular hydrocarbons (CHCs) serve as intraspecific recognition cues (Greene and Gordon 2003; Howard and Blomquist 2005), but also as cues to recognize mutualists (Lang and Menzel 2011; Menzel and Schmitt 2012) or to avoid competitors or predators (Geiselhardt et al. 2011; Mestre et al. 2014; Wüst and Menzel 2017). The composition of substances and compounds of CHC profiles varies between species (Geiselhardt et al. 2011; Menzel et al. 2017). If species use such specific chemical cues for species recognition, they either need to learn or adapt to chemical cues of their interaction partners (Sih et al. 2010).

However, if species recognition is based on general cues (i.e. chemical substances present in many different species) they should be able to recognize non-native predators despite missing co-evolutionary history (Sih et al. 2010). Consequently, the similarity of chemical cues between native and non-native species as well as the species' ability to discriminate between different cue profiles is expected to influence interactions between native and non-native species and can thus contribute to the invasion success of non-native species (Sih et al. 2010).

Ladybeetles have been intentionally redistributed across continents as pest control agents against aphids. In 1982, the multicolored Asian ladybeetle, *Harmonia axyridis*, was introduced to Europe for the biological control of aphids in greenhouses and sold by biological control companies since 1995 (Brown et al. 2007). In 2002, first feral populations were sighted in the Netherlands, Belgium and Germany (Babendreier 2007). Since then, *H. axyridis* has spread over Central Europe and is now a dominant member of the ladybeetle community in many European countries. *H. axyridis* displaces native ladybeetles in Europe but also in North America (Elliott et al. 1996; Koch 2003; Evans 2004; Brown et al. 2011; Roy et al. 2012). The rapid decline in the abundance of native ladybeetle species in the last 30 years has become an increasing cause for concern (Alyokhin and Sewell 2004; Harmon et al. 2006).

So far, research on ecological effects of invasive ladybeetles on native communities has concentrated on intraguild predation among ladybeetles, on prey depletion, on body size and fecundity, on phenology as well as on habitat displacement (reviewed in Roy and Wajnberg 2008). More recently, the role of chemical protection (Kajita et al. 2010; Kajita et al. 2014) and endoparasites (Vilcinskas et al. 2013) of invasive ladybeetles for the invasion success has also been studied. In addition, differences in ant aggression towards native and non-native ladybeetles could contribute to the invasion success of non-native ladybeetles as well (Finlayson et al. 2009). Ants are known as predators, ecosystem engineers and for their important role in communities as hemipteran mutualists (Flatt and Weisser 2000; Stadler and Dixon 2005). Some ant species show aphid tending behavior: They feed on honeydew and defend aphids against various predators (Way 1963; Völkl et al. 1999; Stadler and Dixon 2005). Relying on aphids as food source, ladybeetles are competitors of ants and are thus particularly prone to ant aggression. Reduced intraguild interference with ants is expected to contribute to the invasion success of non-native ladybeetles because of enhanced food access. So far, we lack understanding to which extent ant aggression contributes to the invasion success of *H. axyridis* in Europe (Pell et al. 2008).

Here, we compared the strength of ant aggression towards ladybeetles and ladybeetle reaction upon contact with ants between native and non-native ladybeetles in Europe. In our laboratory experiments, we confronted individuals of four native ladybeetle species (*Coccinella septempunctata*, *Hippodamia variegata*, *Adalia bipunctata*, and *Propylea quatuordecimpunctata*) and the invasive ladybeetle species *H. axyridis* with individuals of two co-occurring native ant species (*Lasius niger* and *Myrmica rubra*). In an additional experiment, we confronted ants with chemically manipulated ladybeetle elytra. The elytra were either untreated, washed and thus expected to be free of chemical cues, or treated with chemical cues of the different ladybeetle species. This dummy experiment allowed us to evaluate the role of chemical cues for the strength of ant aggression. Finally, we analyzed the chemical composition of CHCs using Gas Chromatography-Mass Spectrometry (GC-MS) to compare the similarity of chemical cues among the ladybeetle species used in our behavioral assays. We expected (1) stronger ant aggression towards native ladybeetles compared to invasive ladybeetles; (2) stronger reaction of native ladybeetles compared to invasive ladybeetles if confronted with native ants. In our dummy experiment, we expected (3) weaker ant aggression towards cue-free elytra compared to untreated elytra and (4) comparable ant aggression between initially cue-free elytra with added ladybeetle cues and untreated elytra. Regarding the chemical composition of ladybeetle cues, we expected (5) a species-specific CHC profile for each ladybeetle species included in our experiments.

Methods

Study species

Adult individuals of the five ladybeetle species were collected from field margins in the agricultural landscape of Central Europe (Germany, France and Switzerland) from March till September 2017. Additional individuals of *A. bipunctata* were purchased (Bioinsecte, Adavalue SPRL, Othée, Belgium) as we could not find enough individuals in the wild. Small groups of ladybeetles separated by species were kept in Petri dishes (ø 9 cm) with moistened cotton and stored in a climate cabinet (MLR-352H, Panasonic Corporation, Kadoma, Osaka, Japan; at 20°C, 65 % RH, 16/8 h day night rhythm). The ladybeetles were fed *ad libitum* with pea aphids *Acyrthosiphon pisum*. An initial population of aphids was provided by the Julius-Kühn-Institut (Braunschweig, Germany). Aphids were reared on bean plants *Vicia faba* (variety Sutton Dwarf; Kings Seeds, Essex, UK). Plants had to be renewed twice a week. Both, aphids and plants were kept in climate cabinets (20°C, 65 % RH, 16/8 h day night rhythm).

Ant colonies of *Lasius niger* and *Myrmica rubra* were excavated in meadows and at forest edges in the vicinity of Marburg, Germany. All ladybeetle species co-occurred at the sites where ants were collected. With *H. axyridis* being the most abundant ladybeetle species during the study period and *H. variegata* and *A. bipuncata* being relatively rare at these sites. We always kept two active ant colonies per species with brood during the whole study period. The ant colonies were replaced by fresh colonies in case only a few active workers and/or no brood was present anymore. In total we ended up with 8 ant colonies for both experiments. Each colony was kept in a terrarium (20 cm x 20 cm x 30 cm) in the laboratory. The upper edge of the terraria was covered with Fluon® (Polytetrafluoroethylene dispersion) to prevent the ants from escaping. Water was provided in tubes clogged with cotton and the soil was moistened with a spray bottle twice a week. Ants were fed twice a week with honey and with dead house crickets *Acheta domesticus*.

Aggression experiments with living ladybeetles

The behavioral assays were performed in Petri dishes (ø 9 cm) with Fluon®-covered walls to prevent the ants from escaping. Each Petri dish was only used once in order to prevent contamination with ant or ladybeetle cues. For each trial, three ant workers were caught out of one colony and placed in the Petri dish. After an acclimatization time of 15 min, one adult ladybeetle of the respective species was placed in the center of the Petri dish. During three minutes following elements of ant aggression were quantified: prolonged antennation, opening mandibles, chasing, grasping, biting, and stinging (see Finlayson et al. 2009). Thereof, antennation and opening mandibles were considered as weak aggression (i.e. no contact with ladybeetle and no ladybeetle reaction) and chasing, grasping, biting and stinging were considered as strong aggression. In addition, following elements of ladybeetle reaction were quantified: changing movement direction, retracting legs or antennae, preening, turning on back, flailing legs, fluttering wings, backing, running away and flying away (see Finlayson et al. 2009). Here, changing movement direction, retracting legs or antennae and preening were considered as weak reaction (i.e. no energetically costly increase in movement) were as turning on back, flailing legs, fluttering wings, backing, running away and flying away were considered as strong reaction. If behavioral elements continued for more than three seconds they were counted again. All behavioral assays were recorded with a video camera (LUMIX DMC-FZ300, Panasonic Corporation, Kadoma, Osaka, Japan) mounted on a tripod. The recordings were used for a slow-motion replay if many different behavioral elements occurred very quickly. For the aggression experiments with living ladybeetle individuals, we compared five ladybeetle species in combination with two ant species with at least 20 replicates per combination, resulting in 206 replicates. The order of ladybeetles species identity during the experimental period was randomized.

To compare the strength of ant aggression and ladybeetle reaction between the ant and ladybeetle species, we compared the proportion of strong interactions out of the total counts of interactions observed (see Roulston et al. 2003; Pamminger et al. 2011). First, we calculated a generalized linear model with ant and ladybeetle species identity as well as the interaction between both fixed effects. The model followed quasi-binomial error distribution to account for overdispersion. Since behavioral interactions differed between ant species, differences in interaction strength between the ladybeetle species were analyzed for each ant species separately. In the case of significant overall effects based on a subsequent χ²-test, a Tukey post-hoc test was applied to obtain pairwise contrasts between the ladybeetle species with adjusted p-values. All statistical analyses were conducted using the statistical software environment R version 3.5.2 (R Development Core Team, 2018).

Aggression experiments with manipulated elytra

We used ladybeetle elytra as dummies to manipulate CHCs. Elytra can easily be removed from dead beetles. They contain no secretory glands (Pettersson 2012), and our analyses did not detect any other compounds than CHCs in elytra extracts. Ants can grasp and bite easily into these strongly sclerotized body parts. A number of 60 adult individuals per ladybeetle species plus additional 200 *H. axyridis* individuals were killed in the freezer at approximately -10 °C for 12 h. Thereafter, the dead ladybeetles were defrosted and both elytra were broken off with a clean tweezer. The resulting 120 elytra per ladybeetle species were divided into 80 elytra for the cue extraction and 40 elytra remained untreated. To obtain cue-free ladybeetle elytra, 400 *H. axyridis* elytra were repeatedly immersed in clean 20 ml hexane or dichloromethane (i.e. to solve apolar and polar organic substances): hexane for ten min, dichloromethane for one hour, hexane for one hour, dichloromethane for ten min and again hexane for ten min. Between each step, the elytra were dried on clean paper towel for 30 s. Half of these cue-free elytra were later used as negative treatments (i.e. cues washed away) and the other half as positive treatment (i.e. adding ladybeetle cues of the respective species). To obtain the cues of the different ladybeetle species, 80 elytra of each ladybeetle species were covered with 4.0 ml hexane for ten minutes. Afterwards, the solutions were transferred into smaller vials (4.0 ml, 15 mm x 45 mm) and were stored without cover to allow the hexane to evaporate. The CHCs were then resolved in 800 µl hexane.

In each aggression trial, three ants were confronted with one elytron of either of the following treatments: untreated elytron of the respective ladybeetle species, cue-free elytron of *H. axyridis* with pure hexane (negative treatment), or cue-free elytron of *H. axyridis* with added cues of the respective ladybeetle species solved in hexane (positive treatment). Each elytron of the negative treatment was treated with pure hexane. To this end, the elytron was held with a spring steel tweezer and 10.0 µl hexane was applied on the outer surface with a micropipette. After the hexane had dried, the elytron was turned and another 10.0 µl hexane was applied on the inner surface. For the positive treatment, the same procedure was applied but with the cue solution of the respective ladybeetle species instead of pure hexane. In this dummy experiment, we ended up with 200 replicates with untreated elytra (20 replicates per ladybeetle and ant species combination), 200 replicates of the negative treatment and 200 replicates of the positive treatment (with 20 replicates per cue-donator ladybeetle and ant species combination) resulting in a total of 600 replicates. During the behavioral assays, ant aggression was quantified as described above. Proportions of strong ant aggression were analyzed using a generalized linear model with quasi-binomial error distribution. Treatment, ant-, and ladybeetle species identity as well as their interactions entered the model as fixed effects. Differences in ant aggression between the treatments were compared for each ant species separately (see experiment with living ladybeetles). In case of significant overall effects based on a subsequent χ^2-test, a Tukey post-hoc test was applied to obtain pairwise contrasts between the treatments.

Chemical analysis

CHC extracts for chemical analyses were obtained by immersing the two elytra of one freeze-killed ladybeetle in 1.0 ml hexane for ten minutes. This was done for seven *C. septempunctata*, four *A. bipunctata*, five *P. quatuordecimpunctata*, eight *H. variegata*, and nine *H. axyridis* individuals. All extracts were concentrated under nitrogen flow and injected into a 7890A gas chromatograph coupled to a 5975C mass spectrometer (both Agilent Technologies Inc., Santa Clara, USA) in the split less mode at 250 °C. Helium was used as carrier gas (1.2 ml/min). The stationary phase was a capillary column (Phenomenex Zebron ZB5-HT Inferno, 30 m × 0.25 µm × 0.25 µm). Oven temperature was 60 °C for two min, then increased to 200 °C by 60 °C/min, and then increased to 320 °C by 4 °C/min, where it remained constant for ten minutes. We used an ionization current of 70 eV and scanned molecular fragments from 40 to 650 *m/z*. Data were acquired using the software MSD Chem Station E.02.02 (Agilent Technologies). We analyzed all hydrocarbons with a chain length > C20 and an average abundance of at least 0.5%; the abundance of all hydrocarbons < C20 totaled less than one percent of the total. Substances were identified based on retention time and diagnostic ions. Chemical differences between species were tested with a PERMANOVA (999 permutations, command *adonis*, R-package *vegan,* Oksanen et al. 2013) based on Bray-Curtis distances, which contained the relative abundances of all hydrocarbons as dependent variable in a multivariate analysis.

Results

Ant aggression towards living ladybeetles

The strength of ant aggression differed between ladybeetle species ($\chi^2_{4,196} = 20.61$, $p < 0.001$) and aggression of *L. niger* was stronger than that of *M. rubra* ($\chi^2_{1,196} = 206.49$, $p < 0.001$). We found no interactive effect of ladybeetle and ant species identity on ant aggression ($\chi^2_{4,196} = 2.81$, $p = 0.59$). For *L. niger*, the strength of aggression differed between ladybeetle species (Figure 1a; $\chi^2_{4,96} = 13.16$, $p = 0.01$). Here, aggression was stronger towards *C. septempunctata* compared to *H. axyridis* ($z = 2.99$, $p = 0.02$) and tended to be stronger towards *C. septempunctata* compared to *A. bipunctata* ($z = 2.69$, $p = 0.06$). For *M. rubra*, the strength of aggression differed between ladybeetle species (Figure 1b; $\chi^2_{4,100} = 10.88$, $p = 0.03$). Here, ant aggression was stronger towards *P. quatuordecimpunctata* compared to *A. bipunctata* ($z = 2.95$, $p = 0.03$). We found no further pairwise differences in the ant aggression among the remaining ladybeetle species.

Reaction of ladybeetles to ant encounters

The strength of ladybeetle reaction upon contact with ants differed between ladybeetle species ($\chi^2_{4,196} = 38.00$, $p < 0.001$) and ladybeetle reaction upon contact with *L. niger* tended to be stronger compared to that after confrontations with *M. rubra* ($\chi^2_{1,196} = 3.13$, $p = 0.08$). We found no interactive effect of ladybeetle and ant species identity on ladybeetle reaction ($\chi^2_{4,196} = 4.83$, $p = 0.31$). *Coccinella septempunctata* showed the strongest reaction towards *L. niger* compared to the other ladybeetle species (Figure 1c; effect of species identity: $\chi^2_{4,96} = 24.85$, $p < 0.001$; all pairwise comparisons: $z > 3.67$, $p < 0.01$). For encounters with *M. rubra*, ladybeetle reactions also differed between species (Figure 1d; $\chi^2_{4,100} = 19.31$, $p < 0.001$). Here, ladybeetle reaction was stronger in *C. septempunctata* compared to *H. axyridis* ($z = 2.77$, $p = 0.04$), *A. bipunctata* ($z = 3.94$, $p < 0.001$), *P. quatuordecimpunctata* ($z = 2.85$, $p = 0.04$). In addition, individuals of *H. variegata* tended to react stronger compared to individuals of *A. bipunctata* ($z = 2.68$, $p = 0.06$).

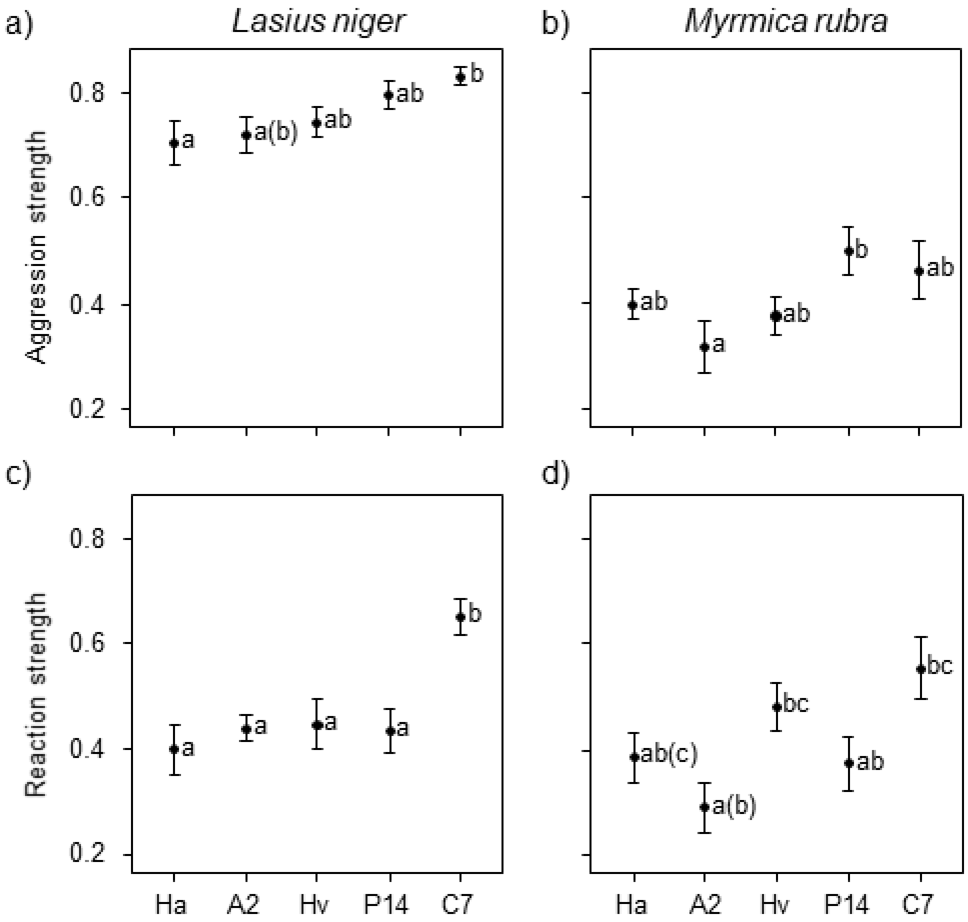

Figure 1. Proportion of strong aggression of (a) *L. niger* and (b) *M. rubra* towards the studied ladybeetle species (Ha: *H. axyridis*, Hv: *H. variegata*, A2: *A. bipunctata*, P14: *P. quatuordecimpunctata*, C7: *C. septempunctata*) and proportion of strong reaction of the five ladybeetle species upon encountering (c) *L. niger* and (d) *M. rubra* (mean ± SE). Different lowercase letters indicate statistical differences (α < 0.05). Letters in brackets indicate statistical trends (α < 0.1).

Aggression towards ladybeetle CHCs

The strength of ant aggression differed between the CHC treatments (Figure 2; $\chi^2_{2,592} = 47.87$, $p < 0.001$) and between the two ant species ($\chi^2_{1,592} = 22.52$, $p < 0.001$). We also found a significant treatment/ant species identity interaction ($\chi^2_{2,592} = 11.86$, $p < 0.01$). Ant aggression did not differ between ladybeetle species or between chemical cues of the different ladybeetle species ($\chi^2_{4,592} = 3.56$, $p = 0.47$) nor did we find a significant treatment/ladybeetle species identity ($\chi^2_{8,592} = 8.71$, $p = 0.37$), ladybeetle species/ant species identity ($\chi^2_{4,592} = 7.35$, $p = 0.12$), and three-way interaction ($\chi^2_{8,592} = 5.10$, $p = 0.75$). For *L. niger*, the strength of aggression differed between the CHC treatments ($\chi^2_{2,289} = 48.90$. $p < 0.001$) but not among ladybeetle species ($\chi^2_{4,289} = 4.18$, $p = 0.38$). Nor did we find a significant treatment/ladybeetle species identity interaction ($\chi^2_{8,289} = 7.55$, $p = 0.48$). Here, ant aggression was weaker when chemical cues were removed compared to untreated elytra ($z = 2.75$, $p = 0.02$). The application of ladybeetle cues of the respective species on cue-free *H. axyridis* elytra increased the strength of aggression ($z = 2.44$, $p = 0.04$) and was similar to the aggression against untreated elytra ($z = 0.38$, $p = 0.92$). Similar to *L. niger,* aggression by *M. rubra* differed between CHC treatments ($\chi^2_{2,285} = 13.84$. $p < 0.001$) but not between ladybeetle species ($\chi^2_{4,285} = 6.19$, $p = 0.19$). Nor did we find a significant treatment/ladybeetle species identity interaction ($\chi^2_{8,285} = 6.37$, $p = 0.61$). Here, ant aggression was weaker when chemical cues were removed compared to untreated elytra ($z = 3.22$, $p < 0.01$). The application of ladybeetle cues of the respective species on cue-free elytra did not restore aggression compared to cue-free *H. axyridis* elytra ($z = 0.81$, $p = 0.69$), hence being lower than towards untreated elytra ($z = 2.48$, $p = 0.03$).

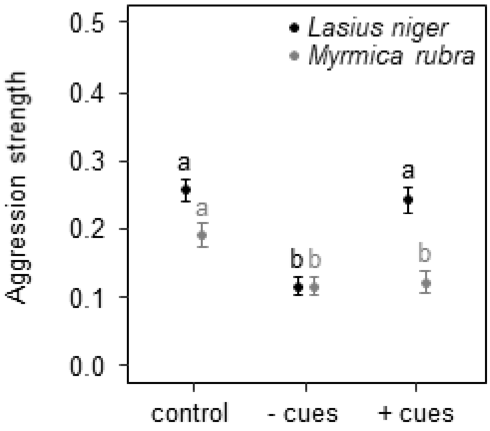

Figure 2. Proportion of strong aggression (mean ± SE) of *L. niger* (black) and *M. rubra* (grey) towards untreated elytra of the studied ladybeetle species (untreated), cue-free *H. axyridis* elytra (- cues), and initially cue-free *H. axyridis* elytra bearing the cues of the respective ladybeetle species (+ cues). Different lowercase letters indicate statistical differences ($\alpha < 0.05$). Letters in brackets indicate statistical trends ($\alpha < 0.1$).

Chemical composition of cues

The composition of the chemical cues differed between ladybeetle species (pseudo-$F_{4,28} = 58.57$, $p < 0.001$) revealing a distinct species-specific chemical profile for each ladybeetle species (see Figures 3 and 4; all pairwise-comparisons: pseudo-$F \geq 41.7$, $p \leq 0.05$). Here, cue composition was relatively similar between *H. axyridis* and *H. variegata* (multivariate distance between species centroids based on Bray-Curtis distances: 0.54). The profiles of both species were characterized by a very high proportion of alkenes, followed by alkadienes and *n*-alkanes. In contrast, the profile of *P. quatuordecimpunctata* was dominated by alkadienes (distance to the *H. axyridis* centroid: 0.73), while the profiles of *C. septempunctata* and *A. bipunctata* strongly differed from all previous species, having high proportions of monomethyl alkanes (as well as di- and trimethyl alkanes in the case of *C. septempunctata*) (distance to the *H. axyridis* centroid for both species: 0.86).

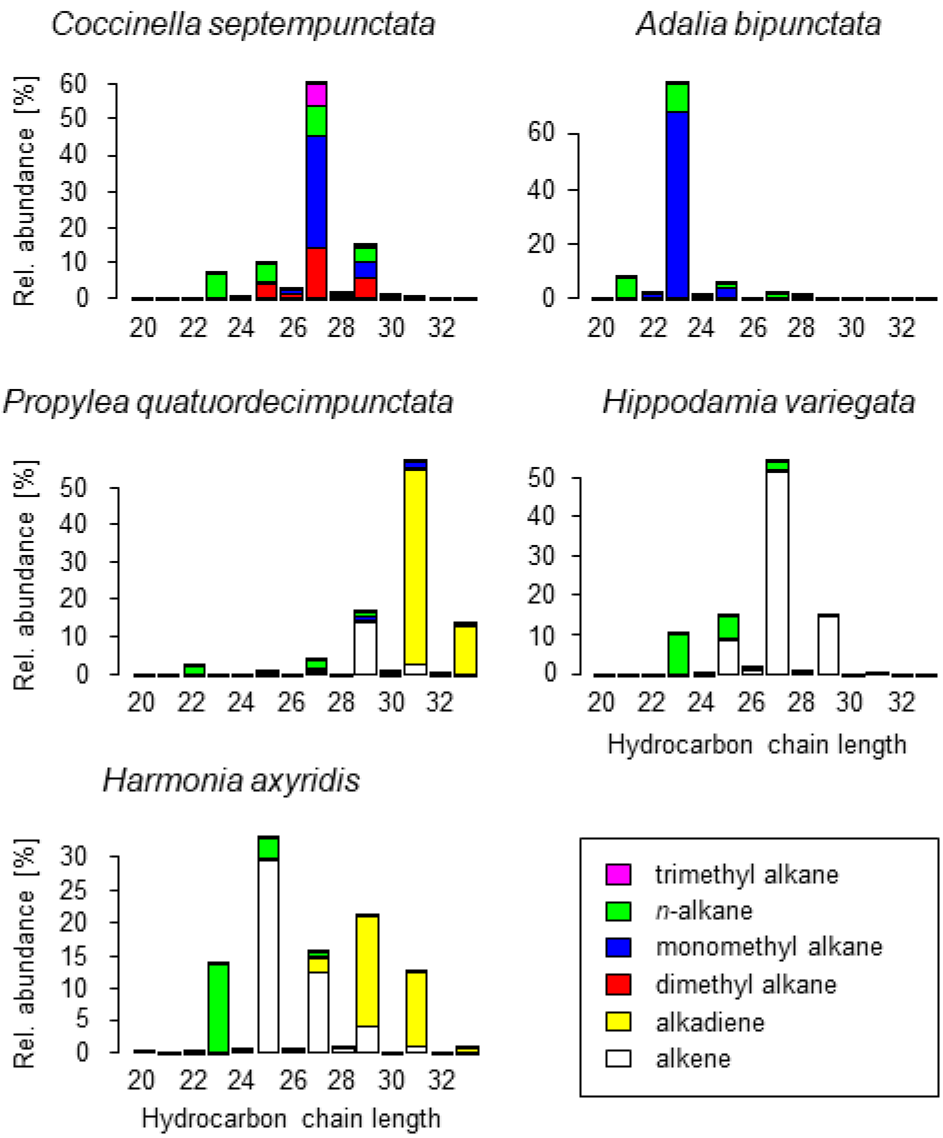

Figure 3. Schematic cuticular hydrocarbon (CHC) profile of the ladybeetle species included in the behavioral assays. The bars represent the relative abundances of different substance classes at different chain lengths. Note that this representation does not show differences between hydrocarbons of the same hydrocarbon class and chain length.

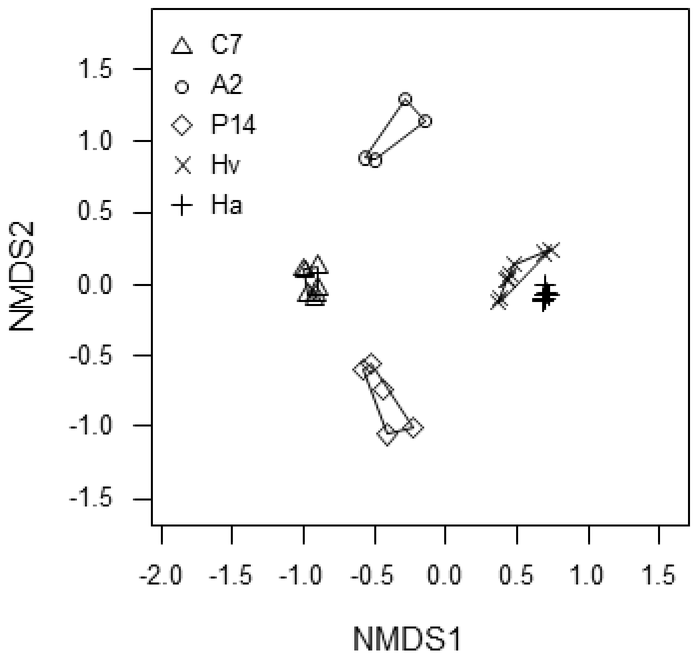

Figure 4. Ordination plot (Non-metric MultiDimensional Scaling with Bray-Curtis dissimilarity) based on the relative abundance of identified substances. Each symbol represents a ladybeetle individual of the respective species.

Discussion

Our behavioral experiments revealed that ant aggression as well as ladybeetle reaction differed between ant and ladybeetle species. Across ladybeetle species, the aggression of *L. niger* was stronger than that of *M. rubra*. In particular, aggression of *L. niger* against *C. septempuncata* and the reaction of *C. septempunctata* upon encounters with *L. niger* were stronger compared to interactions with the invasive *H. axyridis*. Removing chemical cues from ladybeetle elytra reduced aggression of both ant species and the addition of ladybeetle cues on cue-free elytra increased the aggression of *L. niger* but not that of *M. rubra*. In contrast to the behavioral experiments with living ladybeetles, the comparison of ant aggression towards ladybeetle elytra revealed no differences between ladybeetles species identity and their cues. Our chemical analyses showed a distinct species-specific CHC profile for each ladybeetle species, which is a prerequisite for predator-predator naïveté.

Ant aggression and ladybeetle reaction

In terms of effect size, the most evident pattern we observed was the higher aggression of *L. niger* compared to that of *M. rubra*. *L. niger* is known to be more aggressive than *M. rubra* and often dominates *M. rubra* in direct encounters (Binz et al. 2014). In addition, ants of the genus *Lasius* are more strongly involved in trophobiosis with aphids and even overwinter aphids in their nests whereas ants of the genus *Myrmica* regularly prey on other arthropods including aphids (Seifert 2007). Consequently, *L. niger* is expected to be a stronger ladybeetle competitor compared to *M. rubra*.

Differences in ant aggression between ladybeetle species are contrasting and not as simple as we expected. Weaker *L. niger* aggression (fewer grasping and biting) against *H. axyridis* compared to *C. septempunctata* is in line with our expectation. This result suggests that *L. niger* lack the ability to recognize non-native *H. axyridis* as strong competitor and to react accordingly. Further experiments with completely ladybeetle-naïve ants would be required to test whether the recognition ability is learned or innate. If recognition is learned, *H. axyridis* should elicit strong aggression, since it was by far the most common ladybeetle at the sites of ant collection. Furthermore, *H. axyridis* is an equally voracious aphid predator compared to *C. septempunctata* (Ünlü et al. 2020) and consequently an equally strong competitor. Thus, one would expect a selection pressure towards ants being equally aggressive to *H. axyridis* in the long term. Given that many ant workers used in the experiment should have never been in contact with any ladybeetle suggest that ladybeetle recognition may be innate. Ant aggression did not differ between *H. axyridis* and the other native lady beetle species *A. bipunctata, H. variegata* and *P. quatuordecimpunctata*, which are considerably smaller than *C. septempunctata* and hence less relevant ant competitors.

Regarding the role of intraguild interactions in the ant-ladybeetle-aphid system, the vast majority of studies focuses on intraguild predation between ladybeetles (reviewed by Pell et al. 2008), whereas information about differences in ant aggression against ladybeetles is scarce. To our knowledge, the only multispecies comparison including native and non-native ladybeetle species has been conducted by Finlayson et al. (2009). In their study aggression of *M. rubra* workers tending potato aphids *Macrosiphum euphorbiae* was compared between North American, European, and the Asian ladybeetle species *H. axyridis*. Similar to our results, Finlayson et al. (2009) found no differences in the intensity of *M. rubra* aggression against novel *H. axyridis* and co-evolved *C. septempunctata*. Lacking ladybeetle species discrimination in *M. rubra* but not in *L. niger* might again be explained by their involvement in trophobiosis with aphids (i.e. more competitive encounters with ladybeetles), which is more pronounced in the genus *Lasius* than in the genus *Myrmica* (Seifert 2007).

The reaction of the different ladybeetle species towards ants mirrored to some extent differences in the strength of ant aggression among the ladybeetle species, i.e. higher aggression strength led to stronger escape behavior. In particular, *C. septempuncata* showed much stronger reaction if confronted with *L. niger* compared to all other ladybeetle species. Although *L. niger* aggression was strongest against *C. septempuncata*, over proportionally strong reaction suggests that *C. septempuncata* is able to effectively avoid ant aggression. Earlier studies under more natural conditions indicate that *C. septempuncata* is quite successful in avoiding ants (Sloggett et al. 1998) and is better protected with alkaloids compared to *A. bipuncata* (Marples 1993). Regarding interactions with *M. rubra*, *P. quatuordecimpunctata* received highest aggression (possibly due to a stronger habitat overlap) but their reaction was lower compared to those of *C. septempunctata*. *P. quatuordecompuncata* might thus be particularly prone to ant aggression if they feed on ant-tended aphids. In summary, our laboratory experiments revealed stronger aggression of *L. niger* compared to *M. rubra* and provide evidence that *L. niger* aggression as well as ladybeetle reaction is weaker in the invasive *H. axyridis* compared to the equally voracious native ladybeetle *C. septempunctata* – with potential implications for their invasion success in Europe.

Implications of ant aggression for the invasion success of *H. axyridis*

Ants have pervasive effects on terrestrial ecosystems by influencing community structure but also by hampering top-down control (Stadler and Dixon 2005; Sanders et al. 2011). For example, the presence of North American ants reduced soybean aphid predation by *Orius insidiosus* (Anthocoridae) and *H. axyridis* and led to a tenfold increase in aphid numbers (Herbert and Horn 2008). As a result of ant aggression, ladybeetles only feed on ant-tended aphids when untended prey is scarce (Sloggett and Majerus 2000). In the presence of *L. niger*, the invasive *H. axyridis* might outcompete native *C. septempuncata* due to reduced ant aggression and ladybeetle reaction. That it is attacked less by ants – possibly due to predator-predator naiveté – would be an additional competitive advantage for *H. axyridis* compared to its strongest native counterpart. However, experiments under more natural conditions would be necessary to infer competitive advantages of invasive ladybeetles due to release form intraguild interference by ants. Differences in ant aggression between different ladybeetle species could change if ants are actually tending aphids (Way 1963). For example, the myrmecophilous *Coccinella magnifica* received similar ant aggression compared to *C. septempunctat* on trails but only *C. magnifica* fed upon tended aphids (Sloggett et al. 1998). Given the strong context dependency in ant-ladybeetle interactions, we see the need for field or semi-field experiments including ants, ladybeetles, and aphids to compare differences in aphid predation rates among ladybeetle species in presence and absence of ants. Such experiments would ideally include different lifestages of ladybeetles as well as different competing ladybeetle species. Nonetheless, the experimental approach presented here is very well suited to study behavioral responses to native and non-native ladybeetle species since they allow large replicate numbers across multiple species, and enable detailed behavioral analyses. Even though these aggression assays are relatively simple and somewhat artificial, they have been frequently and successfully used to disentangle chemical and behavioral cues underlying aggression in ants (Foitzik et al. 2007; Steiner et al. 2007; Menzel et al. 2009).

The role of chemical cues for intraguild interference

Ants were aggressive against ladybeetle elytra, albeit less so than against living beetles. Removal of chemical cues led to a strong reduction of ant aggression. This indicates that chemical cues are highly relevant for intraguild interactions between ladybeetles and ants. This is not surprising, given that for ants, cuticular chemical cues, such as cuticular hydrocarbons, are the basis for the recognition of nestmates (Howard and Blomquist 2005) and mutualistic interaction partners (Lang and Menzel 2011; Menzel and Schmitt 2012). The addition of ladybeetle cues on initially cue-free elytra led to similar *L. niger* aggression compared to the untreated elytra demonstrating that chemical cues elicit aggression of *L. niger*. Surprisingly, the addition of ladybeetle cues did not increase the strength of aggression by *M. rubra*. The aggression of *M. rubra* was generally lower than that of *L. niger*, which might be due to differences in their food preference (see discussion above). The chemical treatment might further weaken behavioral responses because the cues are not presented in the entirely natural form, and hence trigger weaker or no responses. At least for *L. niger*, aggression could be restored by re-application of CHC extracts reconfirming their role for species recognition. However, in contrast to the live beetles, ant aggression did not differ between different beetle extracts. Hence, the CHCs alone might not fully explain differences in ant aggression, suggesting that behavioral responses by the ladybeetles further modulate ant aggression. Similarly, Finlayson et al. (2009) observed that ant aggression was higher if some ladybeetle species had more exposed body parts on which ants could grasp. Moreover, generally weak ant aggression against elytra might mitigate differences in aggression between ladybeetle species that occur under natural conditions (i.e. against living ladybeetles).

Our chemical analyses of the ladybeetle cues revealed a unique CHC profile for each ladybeetle species. All species were significantly different from each other, with only few shared substances among all ladybeetle species. The shared substances (e.g. *n*-C22, *n*-C23, *n*-C25, and *n*-C27) are commonly found in many insect species and, due to their high melting point, have been related to waterproofing rather than to recognition (Menzel et al. 2019). Notably, the species showed a remarkable chemical diversity even concerning the dominant substance classes: While *C. septempunctata* and *A. bipunctata* profiles were dominated by monomethyl alkanes*, H. axyridis* and *H. variegata* mostly possessed alkenes, and *P. quatuordecimpunctata* mostly contained alkadienes. All of these compounds have vastly different physical properties (Menzel et al. 2019). The adaptive value of these differences still remains to be found. Interestingly, the main compound in our *H. axyridis* samples, a C25-alkene, was not reported in conspecific samples from Japan (Magro et al. 2010), which might be due to between-population differences in this species. Usually, CHC profiles are qualitatively quite invariant within a species (Kather and Martin 2012, Sprenger and Menzel 2020), which is why they are highly useful for chemotaxonomy, i.e. to distinguish closely related or cryptic species. The species-specific chemical profiles also mean that there is no general chemical ladybeetle cue (i.e. substances present on all aphidophagous ladybeetles). Rather, ants have to recognize each species individually, be it by learning or by evolutionary adaptation. From an evolutionary viewpoint, this can be expected if different predators require different antipredator behaviors (Binz et al. 2014; Sadowski and Grosholz 2019). Cue dissimilarity between native and non-native predators as well as species-specific recognition cues are a prerequisite for predator-predator naïveté against invasive predators (Sih et al. 2010). However, a larger species set would be required to formally link chemical cue similarity with aggression strength.

Conclusions

Our behavioral experiments revealed differences in ant aggression and ladybeetle reaction between ant and ladybeetle species. In particular, *L. niger* showed stronger aggression towards ladybeetles compared to *M. rubra,* and aggression of *L. niger* was stronger towards the native *C. septempuncatata* compared to the invasive *H. axyridis*. The strength of this intraguild interference is influenced by the presence of chemical cues on the surface of ladybeetles. The analysis of the chemical ladybeetle cues further revealed species-specific CHC profiles for each ladybeetle species, refuting the existence of a general chemical ladybeetle cue, and making it necessary for ants to recognize ladybeetle species individually. Our aggression assays with *L. niger* thus provide support for the theoretical framework proposed by Sih et al. (2010) suggesting predator-naïveté against novel predators if interaction partners rely on species-specific cues. However, ants might also rely on various and more general cues (e.g. ladybeetle behavior) depending on the context (e.g. if they actually tend aphids). Weaker *L. niger* aggression and ladybeetle reaction likely benefits the non-native ladybeetle *H. axyridis* when competing with the equally voracious native *C. septempunctata* for ant-tended aphids and might ultimately contribute to its invasion success.

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CHAPTER 6

Intraguild interactions between ants and native *versus* non-native lady beetles in North America and the role of chemical cues

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Abstract

Predator-predator interactions may contribute to the invasion success of non-native predators. This may occur due to the lack of co-evolutionary history between predators resulting in reduced aggression towards non-native predators in interference interactions. However, non-native predators might comparably experience similar aggression if they possess cue similarities with native predators.

Here, we tested the aggression strength of two native ant species confronted with native and non-native lady beetles and quantified aggression of ants and the reaction of lady beetles. In an additional experiment, we used lady beetles' elytra to test if species-specific chemical cues elicit different levels of aggression in ants. In both experiments we expected the strongest aggression of ants towards coevolved native lady beetles, intermediate aggression towards non-native, congeneric lady beetles (due to potential cue similarities) and least aggression towards the non-native *Harmonia axyridis*.

We observed differences in strength of aggression and reaction in native and non-native lady beelte-ant interactions. Similarities of ant aggression, but reduced reaction of non-native *H. axyridis* compared to native *Coleomegilla maculata* suggests higher ant tolerance of *H. axyridis*. Furthermore, reaction and aggression strength of non-native *Coccinella septempunctata,* compared to native *Col. maculata* were lower. Additionally, higher aggression strength towards elytra with chemical cues of *H. axyridis* compared to elytra with cues of *C. septempunctata* suggests that *H. axyridis* might be perceived as an increased threat to ant-tended colonies. Cue similarities between congeneric native and non-native species might explain similarities in aggression by ants. Overall, non-native species might benefit from tolerating ant attacks than reduced aggression behavior of ants, facilitating the access to ant-tended resources. This might favour non-native lady beetle species over native lady beetle species in competition on ant-tended aphid resources.

Keywords: intraguild interference; invasion; Coccinellidae; ant aggression; chemical cues

Introduction

Predator-predator interactions can shape prey and predator population dynamics (Sih et al. 1998). For example, if the foraging activity of one predator enhances the predation success of another predator, this interaction will increase the suppression of prey populations (Losey & Denno 1998). Alternatively, prey populations may benefit from antagonistic interactions between predators, such as intraguild predation or predator-induced foraging disruption, which can bring about changes in predator populations (Rosenheim et al. 1995, Polis et al. 1998). Hereby, intraguild interference describes temporally and spatially coinciding predators competing over a shared resource, in which direct predator interactions limit the access to a resource (Putman 1994). These interactions can decrease fitness of the subordinate predator by e.g. aggressive predatory encounters (Eccard & Ylönen 2002).

The effects of non-native predators on native predator and prey species can be significant and can ultimately lead to a loss of biodiversity (Doherty et al. 2016). In particular, non-native predators can destabilize native communities by changing their structure and function (Wagner & Van Driesche 2010). For example, the invasion of the Argentine ant (*Linepithema humile*), altered the native ant community structure (Sanders et al. 2003) and diversity in North America (Human & Gordon 1997). This invasive ant species had further consequences for adjacent trophic levels, e.g. reduced seed dispersal of native plant species by native ants (Bond & Slingsby 1984). The interference with coevolved interaction networks can lead to novel interactions (Verhoeven et al. 2009, Carthey & Banks 2014). Prey species use predator cues as information to assess predation risk of predators (Lima & Dill 1990). The lack of co-evolution between non-native predators and native prey can lead to missing cue recognition and consequently lack of antipredator behaviors by native prey (Banks & Dickman 2007, Sih et al. 2010, Carthey et al. 2017). For example, flightless birds in New Zealand do not flee from invasive mammalian predators, thus suffer heavy predation (Blackwell 2005). Similarly, non-native predators might remain undetected by native competitors in intraguild interactions. Consequently, non-native predators are expected to be stronger competitors for shared prey compared to native predators, which might contribute to their invasion success (Cox & Lima 2006, Banks & Dickman 2007, Sih et. al. 2010). However, if coevolved and non-native predator cues are similar, non-native predators might not benefit from cue novelty by eliciting similar antipredator behaviors as coevolved native predator species (Sih et. al. 2010).

Lady beetles are predators of aphids and coccids and were therefore globally introduced as biological control agents for pest control (Harmon et al. 2007). Invasive lady beetle species are associated with a decline of native lady beetle species, e.g. the Asian *Harmonia axyridis* in Europe and North America (Roy et al. 2016) and the European *Coccinella septempunctata* in North America (Evans 2000). Compared to many native coccinellids, both species are relatively large in body size, highly fecund and voracious (Kajita & Evans 2010, Elliott et al.

1996, Hoki et al. 2014, Ünlü et al. 2020). Moreover, both species are often superior to native lady beetles in intraguild interactions such as resource competition or intraguild predation (Obrycki et al. 1998, Michaud et al. 2002, Aloykhin & Sewell 2004, Ware et. al 2009). Most studies to date focused on intraguild interactions between lady beetle species. However, ants frequently tend and/ or prey on aphids and thus represent competitors for lady beetles (Way 1963, Styrsky & Eubanks 2007). In lady beetle-ant interactions, aggression behavior of ants towards lady beetles, as well as defensive behaviors of lady beetles upon ant attacks can vary in intensity and can be species-specific (see Finlayson et al. 2009, Bucher et al. under review). Chemical cues consisting of cuticular hydrocarbons (CHCs) play a key role for intra- and interspecific communication in ants (Greene & Gordon 2003, Binz et al. 2014, Wüst & Menzel 2017). Previous studies demonstrated, that cuticular hydrocarbons of parasitoid and predator species can serve as interspecific recognition cues, inducing aggressive behaviors in ants (Dettner & Liepert 1994, Pasteels 2007, Bucher et al. under review). Missing cue recognition of non-native lady beetles with distinct chemical cues by ants, might result in lower aggression behavior compared to native lady beetle species. Disturbances in feeding activity of lady beetles by ant aggression can be energetically costly, by reducing time spent for foraging and feeding (Finlayson et al. 2009). Thus, competitive foraging and feeding advantages over native lady beetle species, involving ant-tended prey might contribute to the invasion of non-native lady beetle species (Finlayson et al. 2009).

Here, we tested the strength of intraguild interactions between two native North American ant species, *Lasius neoniger* and *Myrmica americana* and three native North American lady beetle species *Coleomegilla maculata, Coccinella novemnotata* and *Hippodamia convergens,* two non-native congeneric European lady beetle species *Coccinella septempunctata* and *Hippodamia variegata* and the non-native Asian lady beetle species *Harmonia axyridis*. In aggression experiments with living beetles, we expected the highest aggression by ants towards coevolved native lady beetles, intermediate aggression towards non-native, congeneric lady beetles (due to potential cue similarities) and lowest aggression towards non-native *H. axyridis*. We simultaneously assessed lady beetle reaction upon contact with ants. Additionally, we conducted a dummy experiment with chemically manipulated lady beetle elytra (wing covers) to test, whether chemical cues (cuticular hydrocarbons) of lady beetles, can elicit aggression behaviour in ants, serving as recognition cues. In this dummy experiment we compared the strength of ant aggression towards dummy elytra in three treatments: control elytra (species-specific lady beetle elytra), cue-treated elytra (cue-free elytra treated with chemical lady beetle cues) and cue-free elytra. We expected similar aggression towards control and cue-treated elytra and comparably lower aggression towards cue free elytra. When confronted with control and cue-treated elytra, respectively, we expected the highest aggression by ants towards cues of native lady beetles, intermediate aggression towards cues of non-native, congeneric lady beetles and lowest aggression towards cues of non-native *H. axyridis*.

Material and Methods

Study Species

Ants were collected at agricultural field margins at the agricultural research field station of the University of Kentucky (Lexington, Kentucky, USA) from May to June 2018. We excavated ant colonies with brood from the ground and stored them in a terrarium (23.0 × 15.3 × 16.5 cm; *Lasius neoniger*) and buckets (38.1 × 26.67 cm; *Myrmica americana*). *Lasius neoniger* colonies were subsequently maintained in the climate chamber (22 ± 1 °C, light 16 h: dark 8 h.) and *M. americana* colonies were stored in the laboratory under room conditions (25 ± 1 °C, light 16 h: dark 8 h.). We used three *L. neoniger* colonies for the aggression experiments with living lady beetles and two other colonies for the dummy experiments, while the same two *M. americana* colonies were used for both experimental series. Ant colonies were supplied daily with honey and water and fed weekly with freeze-killed crickets and grasshoppers, collected from the research field station. Adult *Col. maculata*, *C. septempunctata* and *H. axyridis* were collected at the location of ant collection (research field station, Lexington, Kentucky, USA) in alfalfa, soybean, and corn fields from May to September 2018. *Hippodamia variegata* was collected from an alfalfa field in Le Roy, IL, USA in May and June. *Hippodamia convergens* was purchased from Rincon Vitova Insectaries, Ventura, CA, USA, in April 2018. *Coccinella novemnotata* larvae were purchased from the Lost Ladybug Project, Cornell University, Ithaca, New York, since no individuals were found in Kentucky. They were kept separately in glass vials until they developed to adults. The adult lady beetles were sorted by species and stored in plastic boxes. They were provided with water and fed *ad libitum* with pea aphids, *Acyrthosiphon pisum* and thawed *Ephestia kuehniella* eggs and kept in climate chambers (22 ± 1°C, light 16 h: dark 8 h). For the second experiment, *H. axyrids* individuals were collected from aggregations on the outside wall of the Department of Entomology at the University of Kentucky (Lexington, Kentucky, USA) in September 2018 and immediately freeze-killed (-7 ± 1°C) in Petri dishes (9.4 × 1.6 cm). Voucher specimens were preserved in Ethanol (70%) and stored under -7 ± 1°C at the Department of Entomology (Animal Pathology Building), at the University of Kentucky.

Aggression experiments with living beetles

Experimental procedure

We used a round Petri dish (9.4 × 1.6 cm) as experimental arena for the ant aggression bioassays. Fluon® was applied on the Petri dish wall to prevent ants from leaving. Three individuals from one ant colony were randomly collected, placed inside the Petri dish and acclimatized for 15 minutes prior to the start of the experiments. A single adult lady beetle was placed into the Petri dish and lady beetle and ant behaviors were quantified over three minutes. Each of the following ant aggression behaviors were quantified: prolonged antennation, opening mandibles, chasing, grasping, biting, and stinging (stinging *M. americana* only; see Finlayson et al. 2009). Aggression behaviors were subsequently divided into weak aggression (prolonged antennation and opening mandibles) and strong aggression (chasing, grasping, biting and stinging). Lady beetle reaction was quantified as following: changing direction of movement, retracting legs or antennae, preening, turning on back, flailing legs, fluttering wings, backing, running away and flying away (see Finlayson et al. 2009). Changing movement direction, retracting legs or antennae and preening were considered as weak reactions and turning on back, flailing legs, fluttering wings, running and flying away were considered as strong reactions. Aggression or reaction behaviors, which lasted longer than three seconds were quantified as new behavior. We compared six lady beetle species in combination with two ant species with at least 19 replicates per species combination, resulting in 243 replicates. All behavioral assays were recorded with a video camera (LUMIX DMC-FZ300, Panasonic Corporation, Kadoma, Osaka, Japan) mounted on a tripod. Videos were analyzed in slow motion, if interactions and behaviors occurred too quickly to be visually quantified during the experiments. Experiments were conducted in the laboratory under 26.8 ± 0.1 °C and artificial lightning.

Dummy aggression experiments using elytra

Preparation of cue-free elytra

The elytra of 240 freeze-killed *H. axyridis* individuals were carefully removed and subsequently collected in a glass vial and stored in the freezer (-7 ± 1°C). After 12 hours elytra were removed from the freezer and transferred into a new glass vial, which was previously cleaned with hexane. We used hexane and dichloromethane to wash off and remove polar and apolar cuticular substances on the elytral surface. We applied 24 ml of hexane (3 times) and transferred it into the glass vial containing the *H. axyridis* elytra. After 10 minutes the hexane solution was removed with a Pasteur pipette. The glass vial was subsequently filled with 24 ml of dichloromethane (2 times). After 10 minutes the dichloromethane solution was removed with a Pasteur pipette. The hexane and dichloromethane washing procedure was alternately repeated with new solvents and during each washing step, the solution was repeatedly, gently mixed. The cue-free elytra were placed in a glass Petri dish and stored in the freezer (-7 ± 1°C), until they were used in experiments.

Preparation of species-specific cue solutions

Lady beetle species were separated into Petri dishes and freeze-killed (-7 ± 1°C). Elytra of lady beetles were carefully removed. At least 120 elytra per species (60 beetles) were collected. Forty elytra were stored in glass Petri dishes as species-specific control elytra (control elytra). The remaining 80 elytra were placed into a separate vial for the preparation of the cue solution and stored for 12 hours in the freezer (-7 ± 1°C). The cue solution was prepared by adding 4 ml of hexane into the vial. After 10 minutes the hexane solution was removed with a Pasteur Pipette and transferred into another glass vial. The cue solution was subsequently left under the fume hood for 24 to 48 hours until the hexane evaporated. The cues were dissolved in 800 µl of hexane, carefully mixed and sealed with a lid. We used micro pipettes with disposable tips to transfer the cue solution on the lady beetle elytra (cue-free *H. axyridis* elytra). Each elytron was slightly lifted with forceps and subsequently treated with 10 µl of cue solution on each side of the elytron, with a drying period of 30 seconds prior to changing sides (cue-treated elytra). The same procedure was applied to the cue-free elytra, with 10 µl applications of hexane per elytron side, which were subsequently used as control elytra (cue-free elytra).

Experimental procedure

Experiments were conducted in round Petri dish dishes (3.5 × 1.0 cm) with a Fluon® cover on the walls, keeping ants inside. Three individual ants were randomly collected from one colony, placed into the Petri dish and acclimatized for 15 minutes prior to the start of the experiments. After one of the randomly assigned treatment elytron (either control, cue-treated or cue-free elytron) was placed in the center of the Petri dish, aggression behavior was quantified for three minutes. Aggression behaviors, lasting longer than three seconds were quantified as new behavior. Prolonged antennation and opening mandibles were classified as weak aggression behaviors, while grasping, biting and stinging were classified as strong behaviors. Ant and elytra treatment combinations were randomized and at least 18 replicates were conducted per treatment and species combination. Overall, we conducted 767 replicates consisting of 255 replicates of the control elytra treatment, 238 replicates of the cue-treated elytra treatment and

274 replicates of the cue-free elytra treatment. All behavioral assays were recorded with a video camera (see above). Experiments were conducted in the laboratory under 25.61 ± 0.13 °C and artificial lightning.

Statistical analysis

We calculated the proportion of strong aggression/ reaction behaviors out of the total frequency of aggression/ reaction behaviors and referred to it as aggression/ reaction strength. To analyse differences in aggression and reaction strength (response variables) respectively, we applied generalized linear mixed models (GLMs) with a quasi-binominal error distribution to account for overdispersion for the living aggression and the dummy experiments, respectively. In the living aggression experiments we tested for differences in strength of aggression and strength of reaction, respectively, of ant and ladybeetle species and their interaction (predictor variables) via ANOVA (χ^2 test). Experiments with *L. neoniger* and *M. americana* were subsequently analyzed separately, i.e. to account for species-specific biological (e.g. morphological) differences. Lady beetle species identity entered the model as predictor variable. The differences between aggression and reaction strength, respectively, between lady beetle species were analyzed via ANOVA (χ^2 test), followed by a post-hoc test (Tukey's contrasts test).

In the dummy experiments we tested for differences in strength of aggression of ant species and dummy treatments and their interaction (predictor variables) via ANOVA (χ^2 test). Based on the biological differences of *L. neoniger* and *M. americana,* we subsequently analyzed aggression of ant species towards dummy treatments separately. Dummy treatment identity entered the model as predictor variable. The differences of aggression strength between dummy treatments were analyzed with an ANOVA (χ^2 test), followed by a post-hoc test (Tukey's contrast test).

Aggression strength towards species-specific control elytra were separately analyzed for both ant species with lady beetle species identity as predictor variable. To test for differences of aggression strength between control elytra we applied an ANOVA (χ^2 test), followed by a post-hoc test (Tukey's contrasts test). Statistical analyzes were conducted by using the statistical software R version 3.5.2 (R Development Core Team, 2018).

Results

Aggression experiments with living beetles

Aggression strength was higher in *L. neoniger* compared to *M. americana* (GLM; $\chi^2 = 14.32$, df = 1, $p < 0.01$) and differed between lady beetle species (GLM; $\chi^2 = 15.35$, df = 5, $p < 0.01$). Additionally, there was an interactive effect of ladybeetle and ant species on ant aggression (GLM; $\chi^2 = 13.36$, df = 5, $p = 0.02$). In *L. neoniger*-lady beetle interactions, *L. neoniger* aggression strength differed between lady beetle species (GLM; $\chi^2 = 16.91$, df = 5, $p < 0.01$). Aggression strength was significantly higher towards *Col. maculata* compared *to C. septempunctata* (Tukey's contrast test (TCT); $p < 0.05$, Fig. 1) and marginally higher towards *Col. maculata* compared to *C. novemnotata* (TCT; $p = 0.08$). Aggression strength did not differ between *H. axyridis* and the remaining non-native and native species (TCT; $p > 0.33$). Aggression between native and non-native *Hippodamia* species (TCT; $p = 0.95$), as well as native and non-native *Coccinella* species did not differ significantly (TCT; $p = 1.00$). In *M. americana*-lady beetle interactions, the aggression strength of *M. americana* towards lady beetle species indicated marginal differences (GLM; $\chi^2 = 10.77$, df = 5, $p = 0.06$). Here, aggression strength was marginally higher towards *C. novemnotata* compared to *C. septempunctata* (TCT; $p = 0.10$, Fig. 1). The aggression strength towards the remaining species was similar (TCT; $p < 0.20$). Reaction strength of lady beetles differed between lady beetle species (GLM; $\chi^2 = 26.13$, df = 5, $p < 0.01$) and was similar upon contact with *L. neoniger* and *M. americana* (GLM; $\chi^2 = 0.10$, df = 1, $p = 0.75$). There was no interactive effect of ladybeetle and ant species on lady beetle reaction (GLM; $\chi^2= 4.74$, df = 5, $p = 0.45$). In interactions with *L. neoniger* , lady beetle reaction strength differed interspecifically (GLM; $\chi^2 = 19.66$, df = 5, $p < 0.01$). *Coleomegilla maculata* reaction strength towards *L. neoniger* was significantly stronger compared to reaction strength of *H. axyiridis* and *C. septempunctata* (TCT; $p < 0.01$ and $p = 0.01$, respectively, Fig. 1) and marginally higher than *C. novemnotata* (TCT; $p = 0.06$). *Harmonia axyridis* reaction strength was similar to the remaining species (TCT; $p > 0.19$, respectively). Both non-native and native congeneric *Coccinella* and *Hippodamia* species respectively, showed similar reaction strength to *L. neoniger* (TCT; $p > 0.95$, respectively). In interactions with *M. americana*, the reaction strength of lady beetle species differed marginally (GLM; $\chi^2 = 10.83$, df = 5, $p = 0.06$). Here, reaction strength varied marginally between *H. axyridis* and *Col. maculata* (TCT; $p = 0.10$, Fig. 1). Reaction strength of the remaining lady beetle species were similar (TCT; $p > 0.25$, respectively).

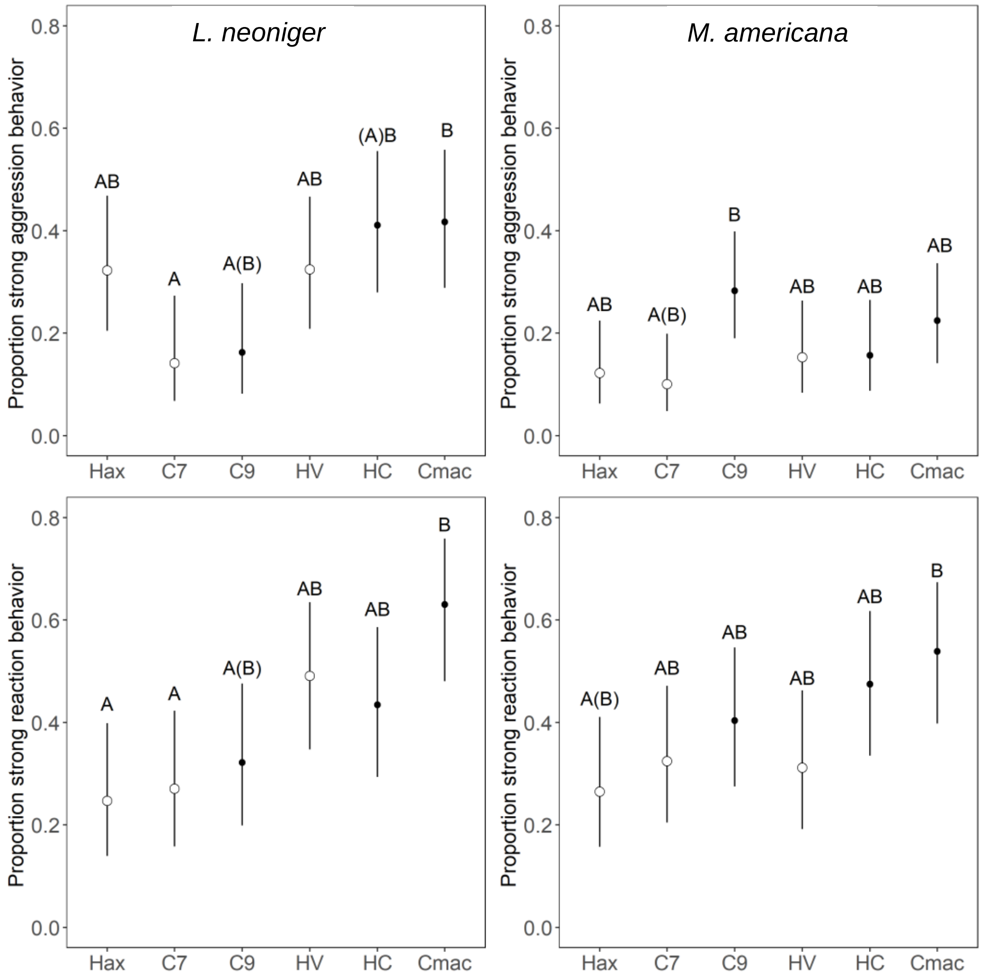

Figure 1. Aggression strength (model prediction ± 95 % CI) of *Lasius neoniger* and *Myrmica americana* confronted with lady beetles and reaction strength (model prediction ± 95 %CI) of lady beetles towards ants of both species (black symbol native, white symbol non-native; Hax = *Harmonia axyridis*, C7 = *Coccinella septempunctata*, C9 = *Coccinella novemnotata* and HC = *Hippodamia convergens,* HV = *Hippodamia variegata*, Cmac = *Coleomegilla maculata*). Different letters indicate statistical differences between species based on Tukey's contrast test ($p < 0.05$).

Dummy aggression experiments

Aggression towards treatment-specific dummy elytra

In these experiments, the aggression strength was similar between ant species (GLM; $\chi^2 = 1.05$, df = 1, $p = 0.31$), but differed between dummy treatments (GLM; $\chi^2 = 42.38$, df = 2, $p < 0.01$). The interaction between ant species and dummy treatments differed marginally (GLM; $\chi^2 = 4.70$, df = 2, $p = 0.10$). *Lasius neoniger* aggression strength towards dummy elytra differed with respect to the dummy treatments (GLM; $\chi^2 = 33.00$, df = 2, $p < 0.01$). Aggression towards cue-treated elytra was significantly lower compared to aggression towards control elytra (TCT; $p < 0.01$, Fig. 2). The cue-free elytra induced less aggression compared to the control elytra (TCT; $p < 0.01$) and marginally less aggression compared to the cue-treated elytra (TCT; $p = 0.08$). *Myrmica americana* aggression strength differed between dummy treatments (GLM; $\chi^2 = 9.97$, df = 2, $p < 0.01$, Fig. 2). Aggression towards cue-treated elytra did not differ from aggression towards cue-free elytra or control elytra (TCT; $p = 0.20$ and $p = 0.40$, respectively). The cue-free elytra induced less aggression compared to the control elytra (TCT; $p < 0.01$).

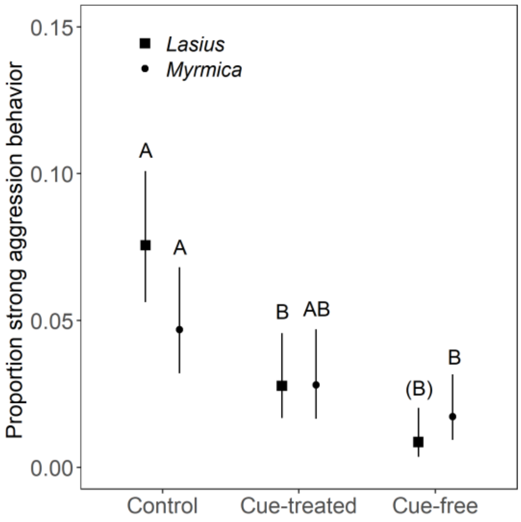

Figure 2. Aggression strength (model prediction ± 95 % CI) of *Lasius neoniger* and *Myrmica americana* confronted with dummy elytra of lady beetles (either control elytra, cue-treated elytra or cue-free elytra). Different letters indicate statistical differences between species based on Tukey’s contrast test ($p < 0.05$).

Agression towards species-specific control elytra

Aggression strength of *L. neoniger* towards control elytra differed among lady beetle species (GLM; $\chi^2 = 19.83$, df = 5, $p < 0.01$). Aggression strength was higher when *L. neoniger* was confronted with *H. axyridis* elytra compared to *C. septempunctata* elytra (TCT; $p < 0.05$, Fig. 3) and marginally higher compared to *C. novemnotata* elytra (TCT; $p = 0.06$), respectively. There, were no species-specific differences in aggression strength of *M. americana* towards control elytra (GLM; $\chi^2 = 7.26$, df = 5, $p = 0.20$, Fig. 3).

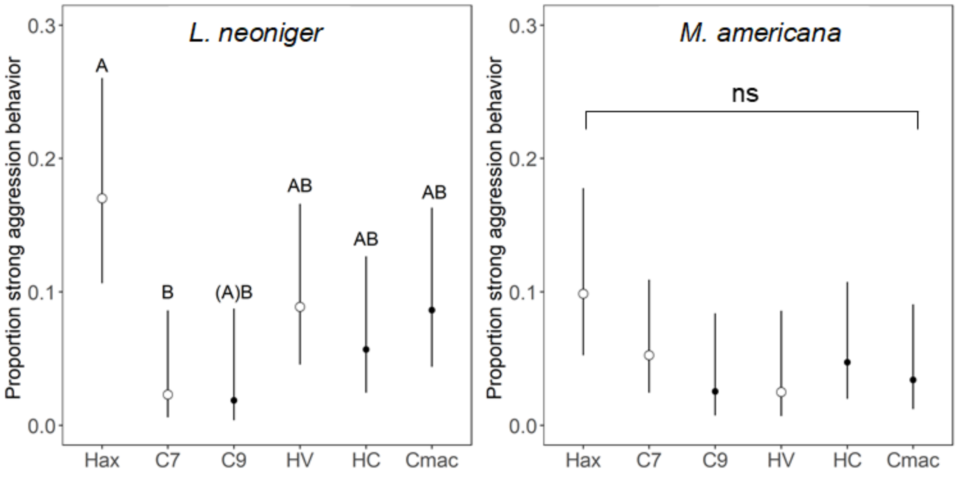

Figure 3. Aggression strength (model prediction ± 95 % CI) of *Lasius neoniger* and *Myrmica americana* confronted with species-specific control elytra of the different lady beetle species (black symbol native, white symbol non-native; Hax = *Harmonia axyridis*, C7 = *Coccinella septempunctata*, C9 = *Coccinella novemnotata* and HC = *Hippodamia convergens,* HV = *Hippodamia variegata*, Cmac = *Coleomegilla maculata*). Different letters indicate statistical differences between species based on Tukey's contrast test ($p < 0.05$).

Discussion

Our findings revealed differences in lady-beetle ant interactions between ant species, as well as native and non-native lady beetle species. The aggression behavior towards lady beetles was higher in *L. neoniger* compared to *M. americana*. In the aggression experiments with living beetles, *L. neoniger* exhibited higher aggression towards the native *Col. maculata* compared to the non-native *C. septempunctata.* Moreover, in *L. neoniger* interactions, the reaction of native *Col. maculata* was higher compared to that of the non-native *H. axyridis* and *C. septempunctata*. *Lasius neoniger* and *M. americana* aggression was similar towards the remaining native and non-native lady beetle species, respectively. Moreover, *Lasius neoniger* and *M. americana* were aggressive to species-specific control elytra of lady beetles. Interestingly*, L. neoniger* showed higher aggression strength towards *H. axyridis* elytra than towards *C. septempunctata* elytra.

Aggression experiments with living lady beetles

Aggression strength towards living lady beetles was lower in *M. americana* compared to *L. neoniger*. Consistent with our findings, in Europe, differences in aggression strength of native ant species of the genera *Myrmica* and *Lasius* confronted with lady beetles, demonstrated lower aggression of *Myrmica rubra* compared to *Lasius niger* (Bucher et al. under review). In contrast to *L. neoniger*, aggression of *M. americana* did not significantly differ between lady beetle species, suggesting that this species is less likely to distinguish between lady beetle species. Similarities in aggression behavior can be explained by, shared general cues (e.g. visual cues, behvioral cues) of native and non-native lady beetle species, inducing similar aggression behavior in ants (Sih et al. 2010). Thus, in direct confrontations with lady beetles, ants exhibit aggression behavior, regardless of lady beetle origin.

In *L. neoniger* interactions, we found differences in strength of aggression and reaction between native and non-native lady beetles. Our findings suggest that differences in interaction strength could be explained by species-specific ant aggression tolerance (Majerus et al. 2007). Native *Col. maculata* might be less tolerant to ant aggression than non-native *H. axyridis*, given that both experience similar aggression*,* but the reaction of *H. axyridis* is comparably lower. Previous research on lady beetle-ant interactions demonstrated that fire ants (*Solanopsis invicta*) were more effective in aggressively forcing *H. convergens* from plants than *H. axyridis*, indicating higher tolerance of *H. axyridis* towards this ant species (Dutcher et al. 1999). Thus, ant tolerance of *H. axyridis* might facilitate access to ant-tended aphid prey (Pell et al. 2008). Similarly, to *H. axyridis*, non-native *C. septempunctata* elicited lower aggression strength and additionally showed lower reaction strength in comparison with native *Col. maculata*. In

Europe, *C. septempunctata* coexists with *Formica rufa* and consequently has shown intermediate ant tolerance in prior studies in its native range (Sloggett & Majerus 2000). In our study, however, *C. septempunctata* reaction strength might be low due to a reduced aggression strength by ants and therefore it is unclear, if this species can tolerate high ant aggression strength beyond its native range. Lower aggression strength by ants towards *C. septempunctata* could be further explained by e.g. species-specific chemical protection of *C. septempunctata* to deter predators, since reflex bleeding of *C. septempunctata* was observed in ant interactions (Bhatkar 1982). Interestingly, both native and non-native *Coccinella* species experience similarly low aggression behavior, which could be explained by similarities in cues or chemical protection of these congeneric species. The ant tolerance of non-native *H. axyridis* and *C. septempuncata* might result in a competition advantage in exploiting ant-tended resources compared to non-native lady beetle species, such as *Col. maculata.* Differences in foraging interruption of ants by aggressive attacks, might result in differences in food uptake and therefore oviposition behaviour by lady beetles (Takizawa & Yasuda 2006). Thus, besides predation advantages, both non-native species might benefit from fitness advantages, however this requires further research under more natural conditions.

Aggression experiments with chemically manipulated lady beetle elytra

Strength of aggression towards lady beetle elytra suggests that chemical cues contribute to the aggressive behavior of ants. Our findings are consistent with a similar study in Europe, demonstrating that ants react aggressively towards lady beetle elytra (Bucher et al. under review). In our study, species-specific elytra elicited higher aggression strength, than the cue-treated elytra. This could be explained by a decreased signalling effect of cuticular substances re-applied on lady beetle elytra (cue-free) compared to species-specific elytra. During the chemical application of the cue solution, cues might not have been equally distributed on the elytron, due to inconsistencies in adherence of cues to the elytral surface. Thus, the untreated species-specific elytra might be more consistently covered in cuticular cues, than the cue-treated elytra. Although cues of lady beetles are species-specific (Bucher et al. under review) the identity of cuticular substances (substance mixtures) inducing ant aggression remains unknown. Ants can discriminate between nestmates and hetero- and conspecifics; therefore, ant recognition is based on a comparison of colony-specific chemical cues, particularly cuticular hydrocarbons, as an internal template to the individual cuticular hydrocarbon profile of the cue donor (Sturgis & Gordon 2012). Thus, threshold-depending cue dissimilarities between the internal template and the received cues can mediate behavioral response, such as aggression behavior in ants (reviewed in Sturgis & Gordon 2012). Previous research on parasitoid-ant interactions, e.g., demonstrated that aphid parasitoids acquire ant hydrocarbons through direct contact to ants, manipulating their chemical profile by chemical camouflage, to prevent ant aggression (Takada & Hashimoto 1985, Akino &Yamaoka 1998, Akino 2008). However, another study on aggression behaviour of ant colonies to congeneric ant species, showed, that cue dissimilarities do not always mediate aggression behaviour; particularly, aggression behavior towards a co-occurring ant species was higher, compared to an allolopatric ant species. This indicates that some ant species might have developed enemy-specific recognition and behavioral response (aggression or defense) towards significant, coevolved enemies (Scharf et al. 2011). According to our expectations, aggression strength towards non-native and native *Coccinella* species, as well as between both *Hippodamia* species, were similar. Magro et al. demonstrated (2010) that CHCs of closely related lady beetle species tend to be more similar compared to cues of more genetically distantly related species. Thus, similar cues of congeneric lady beetle species might explain similar aggression strength of ants, regardless of lady beetle origin. Moreover, our findings reveal that strength of *L. neoniger* towards *H. axyridis* elytra was higher compared to aggression strength towards *C. septempunctata* elytra, suggesting species-specific discrimination of chemical lady beetle cues by *L. neoniger*. In North America, *C. septempunctata* started dominating the landscape in the 1980s followed by *H. axyridis* in the 1990s (Harmon et al. 2007, Brown & Miller 1998, Koch et al. 2003). Brown and Miller (1998) further showed that *H. axyridis* dominated the coccinellid landscape including the previously dominant non-native *C. septempunctata* in apple orchards in North America. Differences in aggression could be explained by enemy-specific risk posed by the predator (Scharf et al. 2011). Should *C. septempunctata* show comparably lower interactions with ants in the invaded range, *H. axyridis* might be the dominating non-native lady beetle species, engaged in competitive interactions with ants. The interaction between ants and *C. septempunctata* and *H. axyridis* beyond their native range requires further research (Pell et al. 2008). Especially if predation pressure on aphids by *H. axyridis* reduces resource availability for ants (honedew), ants might have developed high aggression towards *H. axyridis* within the time of its introduction to North America. Future field studies on ant-lady beetle interactions involving non-native *H. axyridis* and C. *septempunctata*, might shed light on competitive advantages of non-native lady beetle species on ant-tended aphids and their effects on the native lady beetle community.

Conclusion

Our findings on lady beetle-ant interactions show that ant aggression and lady beetle reaction can differ between native and non-native lady beetle species. In interactions with *L. neoniger,* non-native *H. axyridis* showed reduced reaction behavior upon similar ant aggression compared to native *Col. maculata.* Additionally, non-native *C. septempunctata* received reduced aggression of *L. neoniger* and showed lower reaction compared to native *Col. maculata.* The chemical cues on lady beetle elytra contributed to the aggression behavior of ants. Moreover, *L. neoniger* was more aggressive towards elytra of *H. axyridis* than of *C. septempunctata*. Overall, we show that ant aggression towards non-native *H. axyridis* is similairly high compared to native lady beetles, suggesting that this species does not benefit from reduced aggression in its invaded range. The lower reaction behavior compared to native *Col. maculata,* suggests that *H. axyridis* might benefit from a comparably higher ant tolerance. Further research is required to provide insights on ant tolerance of non-native lady beetle species, which could reveal potential effects on resource competition with native lady beetles.

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CHAPTER 7

General Discussion

Ayse Gül Ünlü

Summary

This thesis aims to contribute to the naïveté research among terrestrial arthropods which received relatively little attention up to date (Cox & Lima 2006, Carthey & Banks 2014). Our model systems consist of native and non-native lady beetles interacting with the native pea aphid *Acyrthosiphon pisum* and native ants of the genera Myrmica and Lasius in Europe and North America. To assess naïveté, we focused on antipredator responses of *A. pisum* in predator-prey interactions and aggression behavior of ants in predator-predator interactions towards non-native and native lady beetles, as well as their chemical cues. In addition, we quantified aphid consumption of lady beetles to assess their voracity. In North America, our lady beetle species set included non-native and native lady beetle species from the same genera, expecting similar chemical cues within lady beetle genera (Magro et al. 2010). We analyzed the chemical composition of cuticular hydrocarbons using Gas Chromatography-Mass Spectrometry to compare the similarity of chemical cues among lady beetle species. Our comparisons of native and non-native species interactions revealed differences between non-native lady beetle species and native *A. pisum*. In Europe, *A. pisum* did not show any avoidance behavior towards chemical cues of non-native *H. axyridis*, but towards native lady beetle species. In North America, all chemical cues of lady beetle species were avoided by *A. pisum*, regardless of origin. The body size of lady beetles and aphid predation rates were positively correlated. *Harmonia axyridis* and C*. septempunctata* were the largest and most voracious lady beetle species in North America and in Europe. In Europe, a more natural set-up revealed that *A. pisum* avoids cues of the largest native *C. septempunctata* but shows no avoidance behavior towards chemical cues of non-native *H. axyridis* or smaller native lady beetle species. Moreover, dropping behavior of aphids was similar between native lady beetle species and non-native *H. axyridis*. In addition to predator-prey interactions, we found differences between native and non-native lady beetle species in intraguild interactions with ants. In Europe, ant aggression and lady beetle reaction were higher in native *C. septempunctata* compared to non-native *H. axyridis.* In North America, ant aggression was similar between *H. axyridis* and native lady beetle species. Furthermore, ant aggression was higher towards the native *Col. maculata* compared to the non-native *C. septempunctata*. Additionally, the reaction of native *Col. maculata* towards ants was comparably higher than the reaction of non-native *H. axyri*dis and *C. septempunctata*. The cuticular chemical cues of lady beetle species in Europe were species-specific in their composition. We found that ants showed aggression behavior towards cuticular chemical cues on lady beetle elytra. The intercontinental comparison revealed that lady beetle-aphid and lady beetle-ant interactions differed in Europe and North America. While the European population of *A. pisum* did not avoid chemical cues of non-native *H. axyridis*, the North American *A. pisum* population showed avoidance behavior towards all non-native lady beetle species, including the non-native

H. axyridis. In Europe, ant aggression towards non-native *H. axyridis* was lower compared to native *C. septempunctata,* whereas in North America the aggression behavior of ants was comparably similar between non-native *H. axyridis* and native lady beetle species.

Prey response varies with cue type and cue similarities

Predator cues can serve as sensory information for either prey or other predators (Lima & Dill 1990). Our findings shed light on differences in prey and predator response upon confrontation with native and non-native predators or their chemical cues. In predator-prey interactions, predator presence serves as information for immediate predation risk. Accordingly, dropping behavior of aphids is an efficient response to escape immediate predation risk implied by lady beetles (Losey & Denno 1998). In the dropping experiments, *A. pisum* dropping did not differ between native lady beetles and non-native lady beetles. This indicates that cues involved in lady beetle presence, eliciting dropping behavior in *A. pisum* are general predator cues, e.g. behavioral cues, which non-native and native lady beetle species have in common. Moreover, dropping is induced by the release of an alarm pheromone, which is a general response of aphids, attacked by predators, such as lady beetles (Losey & Denno 1998). Thus, lady beetle presence implies an increased risk of predation and involves general cues inducing aphid dropping, regardless of lady beetle origin (Carthey & Banks 2014, Sih et al. 2010). Similarly, ants confronted with present lady beetles behaved aggressively, regardless of lady beetle origin. However, aggression behavior of ants differed between native and non-native lady beetles. In the dummy experiments we demonstrated that cuticular substances contribute to ant aggression. We showed that the composition of these cuticular substances on lady beetle elytra are species-specific. This indicates that in addition to shared general lady beetle cues species-specific chemical cues of lady beetles are involved in ant aggression. In contrast to immediate risk implied by predator presence, remaining chemical cues of predators can persist in the environment and indicate potential predation risk (Kats & Dill 1998). In contrast to predator presence, chemical cues of absent predators imply lower predation risk and accordingly the induced antipredator behavior of prey requires less energy (Lima & Dill 1990). In our leaf-choice and plant-choice experiments we showed that aphids can avoid chemical cues of lady beetles. Prior studies have revealed that the chemical cues in lady beetle footprints serve as signals for nearby-predator presence, implying potential predation risk for aphids and psyllids (Ninkovic et al. 2013, Seo et al. 2018b). Moreover, the cuticular hydrocarbon profiles of lady beetles are species-specific and as such the footprints left on plant tissues (Kosaki & Yamaoka 1996, Geiselhardt et al. 2011). Based on our findings in Europe, *A. pisums* shows missing avoidance behavior of chemical cues of *H. axyridis* in the leaf-choice and plant-choice experiments. This suggests that *A. pisum* does not associate chemical cues of *H. axyridis* with predation risk, lacks efficient response and is therefore naïve towards predator-specific chemical cues of *H. axyridis*. Thus, chemical cue novelty of non-native predator species can result in an inefficient antipredator response and lead to a potential predation advantage over non-native species (Sih et al. 2010). Interestingly, native and non-native lady beetle species from the same genus induced similar aphid or ant response, which could be explained by cue similarities (Magro et al. 2010). The analysis of the CHC composition of lady beetles in North America will provide us with supportive information on cue similarities between congeneric native and non-native lady beetle species and will be included in the fifth manuscript prior to publication.

Prey response varies with predation risk

The plant-choice experiments revealed cue avoidance behavior towards native *C. septempunctata,* but not towards the smaller native lady beetle species and non-native *H. axyridis.* In contrast to the leaf-choice experiments, feeding disturbances of *A. pisum* by increased searching behavior on neighboring plants can cause more severe energy constraints, which can result in reproductive costs (Lima & Dill 1990, Nelson 2007). This indicates that the avoidance behavior of *A. pisum* might be influenced by antipredator behavioral flexibility driven by the trade-off between energy intake and the risk of predation implied by the chemical cues of lady beetles (Dill & Ydenberg 1986, Lima & Dill 1990). Our findings suggest that the implied predation risk of *C. septempunctata* is higher compared to the remaining native lady beetle species, since *C. septempunctata* is comparably more voracious. In contrast to the chemical cues of smaller lady beetle species, chemical cues of *C. septempunctata* might signalize a higher predation risk and consequently outweigh the benefits of feeding on the present plant and ultimately result in a change of host plants (Lima 1998).

Naïveté – an evolutionary snapshot?

Time since introduction is a key determinant for naïveté, as prey naïveté declines with the number of generations since introduction (Anton et al. 2020). After numerous prey generations, prey can adapt efficient antipredator behaviors towards predators (Yoshida et al. 2003, Anton et al. 2020). An intercontinental comparison provided us with a snapshot of non-native predator-prey interactions in Europe and North America; while the European study represents a snapshot of initial interactions with *A. pisum* and non-native *H. axyridis*, the North American study portrays a snapshot of prolonged interactions between *A. pisum* and non-native *H. axyridis.* Our differences in non-native predator response of *A. pisum* might derive from differences in interaction frequencies and time since introduction of non-native lady beetles, which has been longer in North America than in Europe. Thus, we suggest, that the shared experience between the North American *A. pisum* population and non-native *H. axyridis* might have resulted in rapid adaptations of antipredator behaviors in prey. Agricultural pest species evolve often rapidly towards environmental and human-induced selection pressures (Pélissié et al. 2018). To enhance our comprehension of evolutionary (adaptation) as well as ecological changes (competition, dispersal) in non-native predator-prey interactions, long-term studies are required, starting with the initial introduction of the non-native predator (Mallon et al. 2015, Anton et al. 2020). Overall predator-predator and predator-prey naïveté might decline over time, due to adaptations of the native community and the tremendous impacts of successful invaders might decrease (Yoshida et al. 2003, Anton et al. 2020). Evolutionary changes not only occur in native communities but also in the invader itself, as the non-native predator is exposed to new biotic and abiotic factors in the native community (Novak 2007). Studies in the native range of invasive lady beetles are highlighted as ‘snapshot of the ancestral lady beetle’ and could be compared to studies in the invaded range to reveal evolutionary changes (Sloggett 2012). Overall, further studies in the invaded range of non-native predators will provide insights into the evolutionary changes involved in non-native predator-prey interactions and their ecological effects, whereas studies in the native range will provide baseline information for evolutionary changes in the non-native predator itself (Reznick & Ghalambor 2001, Sloggett 2012).

Invasion success and body size

Non-native species possess traits which contribute to their establishment success (Baker & Stebbins 1965, Sax & Brown 2000). These traits are characteristic to the native range of non-native species and have preadapted them to successfully establish in new regions (Baker & Stebbins 1965, Mack 2003, Alzate et al. 2020). Non-native *H. axyridis* in Europe and North America, as well as non-native *C. septempunctata* in North America have preadapted life-history traits (Slogget 2012, Evans et al. 2011); among these larger body size compared to native lady beetle species is suggested to have promoted their establishment success (Eliott et al. 1996, Kajita & Evans 2010, Hemptinne et al. 2012). Large body size in lady beetles is correlated with high fecundity and can additionally indicate a reproductive advantage over smaller non-native lady beetle species (Kajita & Evans 2010). Not only potential reproductive rate but also speed of movement are positively correlated with body size (Hemptinne et al. 2012). Higher population densities by increased offspring production can lead to dispersal and expansion of the current range (Davis 2009). In addition, our findings show that increasing body size of lady beetles correlates with higher predation rates. Previous studies have demonstrated that *H. axyridis* can dominate over native lady beetles in exploiting shared resources, benefitting e.g. from larger body size and predatory efficiency (Michaud 2002, Crookes et al. 2019). Similarly, Hoki et al. (2014) showed that *C. septempunctata* has higher consumptive effects and is additionally larger in body size compared to native *C. novemnotata* in its invaded range. In addition, Bahlai et al. (2015) found that native *Col. maculata* and non-native *A. bipunctata* have declined, while co-occurring with non-native *H. axyridis* and *C. septempunctata* over a time period of 24 years in southwestern Michigan. This study suggests that the decline of native *Col. maculata* and non-native *A. bipunctata* is likely due to a niche overlap with larger non-native lady beetles resulting in competitive exploitation. In addition to competition on shared food resources, *H. axyridis* and *C. septempunctata* are successful intraguild predators of native coccinellids in their invaded range (Snyder et al. 2004, Aloykhin & Sewell 2004, Pell et al. 2008, Ware et. al 2009, Moser & Obrycki 2009). Competition in the same environment can result in the displacement of native lady beetle species, if non-native lady beetle species are comparably superior in survival and acquiring shared resources (Harmon et al. 2007, Snyder 2009, Bahlai et al. 2015). Our findings demonstrate that *H. axyridis* and *C. septempunctata* consumed more aphids compared to smaller native lady beetle species in a limited time frame, indicating enhanced foraging abilities and therefore a competition advantage. Overall, a relatively large body size compared to native lady beetle species is an important predictor for the establishment and invasion success of non-native lady beetle species (Hemptinne et al. 2012).

Further research options

Our study provides insights into the research on predator-prey naïveté among insect species. In particular, we revealed that missing cue avoidance behavior towards chemical cues of non-native *H. axyridis*, indicates prey naïveté in Europe. The aphid population used for the European experiments were collected prior to the establishment and spread of *H. axyridis* (Brown et al. 2007) in Europe and subsequently maintained as a colony in the laboratory. In comparison to the pea aphids used for our experiments, wild pea aphid populations experienced high densities with *H. axyridis* in the field, given that European establishments of *H. axyridis* were reported in the late 1990s and this species rapidly spread (Brown et al. 2007). To develop a full picture on the potential decline of predator-prey naïveté over time, future studies should examine, if wild European *A. pisum* populations have adapted avoidance behavior towards chemical cues of the currently invasive *H. axyridis*.

Our findings revealed differences in native and non-native lady beetle-aphid and lady beetle-ant interactions. Non-native lady beetle species received less aggression and/or showed lower reaction towards ants. To understand if non-native lady beetle species are tolerant to ant attacks in their invaded range, lady beetle-ant-aphid interactions should be examined (Dutcher et al. 1999, Layman & Lundgren 2016). For example, a competition experiment could be conducted with an ant-tended aphid colony on single plants with either a single adult lady beetle or a lady beetle larva. Attack rates of ants towards lady beetles can be quantified and compared to provide information on differences between aggression levels towards native versus non-native lady beetle species. Moreover, aphid consumption of lady beetles can be quantified and compared between native and non-native lady beetle species, showing the potential of exploiting ant-tended aphid colonies. Both aggression levels and consumption rates can provide information on ant tolerance of lady beetles. As for field observations, aphid tended colonies can be observed and lady beetle presence and activity in these colonies documented to reveal differences between native and non-native lady beetle species in interactions with ants (Sloggett & Majerus 2000, Majerus et al. 2007). These experiments could enhance our understanding of competition on shared resources between ants and lady beetles, as well as competition between lady beetle species of different origins. Furthermore, these experiments could reveal, if ant-tolerant non-native lady beetles can benefit from an enemy-free space in the ‘safe’ vicinity of ants (Pell et al. 2008). Specifically, ant tolerant non-native lady beetle species could benefit from reduced intraguild predation, if their predators, such as other lady beetle species, were less tolerant to ant attacks (Pell et al. 2008). Overall, this could provide us additional information on the role of ant tolerance in the establishment success of non-native lady beetle species.

Chemical cues of predators, inducing avoidance behavior have a repellent effect on herbivores and can additionally decrease oviposition (Herrmann & Thaler 2014, Seo et al. 2018b). Additionally, chemical cues of predators can reduce the reproduction of herbivores, deriving from feeding inhibitions and an extensive search for suitable feeding and oviposition sides (Herrmann & Thaler 2014, Nelson 2007). Based on our findings, the chemical cues of lady beetle species induced avoidance behavior in *A. pisum* on broad bean plants. Similarly, a prior study showed that the bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus) detected and avoided barley leaves bearing lady beetle footprints of *C. septempunctata* via chemicals in the lady beetle tracks (Ninkovic et al. 2013). Moreover, the Asian citrus psyllid, *Diaphorina citri* (Kuwayama) avoided footprints of larvae and adults of *H. convergens* on citrus leaves (Seo et al. 2018 a, b). Lady beetle chemical cues have therefore a promising potential to be used in the control of herbivore pest species on crops. Future research must examine which substance mixtures of chemical lady beetle cues are involved in the avoidance behavior of prey. After the detection of the required substances, these can be synthetically produced and made applicable for use as herbivore repellant in crops. Chemical cues might have an intra-specific signaling effect, for example larval tracks of lady beetles serve as an oviposition-deterring pheromone in conspecific females (Doumbia et a. 1998, Hemptinne & Dixon 2000). The evaluation of the repellant must therefore consider potential negative effects on non-target species. Specifically, predators from which the cue derived from might be affected. Overall, chemical cues of predators might have a potential as applicable repellents for herbivores on crops, however non-target effects due to potential intra-specific signaling in the predator itself, must be considered and evaluated.

Conclusion

This thesis provides valuable insights on invasion mechanisms of non-native lady beetles on two continents by revealing differences in native and non-native lady beetle-aphid and lady beetle-ant interactions. Our findings on lady beetle-aphid interactions in Europe represent a snapshot of the initial phase of non-native predator-prey interactions, whereas our findings in North America portray non-native predator-prey interactions existing for a comparably longer time. In Europe and in North America, *A. pisum* avoided chemical cues of coevolved lady beetle species. However, missing avoidance behavior towards chemical cues of the non-native *H. axyridis* indicates an inefficient antipredator response and thus predator-prey naïveté of *A. pisum* in Europe. In contrast, the North American *A. pisum* population avoided non-native *H. axyridis* cues as well as cues of non-native lady beetles of European origin. Aphid consumption of lady beetles was positively correlated with body size. *Harmonia axyridis* and C*. septempunctata* were the largest and most voracious lady beetles on both continents. In contrast to Europe, ants in North America experienced high densities of non-native lady beetles over a longer time. The lady beetle-ant interactions revealed differences in aggression and reaction between native and non-native lady beetle species on both continents. In Europe, native *C. septempunctata* received higher aggression and showed stronger reaction behavior towards ants in comparison to non-native *H. axyridis*. Similarly, in North America native *Col. maculata* received higher aggression and showed stronger reaction behavior compared to non-native *C. septempunctata*. This indicates that non-native lady beetle species can receive less ant aggression than native lady beetle species in the invaded range. In contrast to Europe, in North America aggression behavior between native lady beetle species and non-native *H. axyridis* was similar, whereas the reaction behavior of non-native *H. axyridis* was lower compared to native *Col. maculata*. A reduced reaction of non-native *H. axyridis* despite of high ant aggression might indicate increased ant tolerance. In Europe, our chemical analysis of cuticular hydrocarbons showed that the CHCs profile of lady beetles is species-specific. Moreover, our findings demonstrate that ants can show aggression behavior towards cuticular chemical cues on lady beetle elytra. The intercontinental differences in non-native species interactions might derive from differing levels of experience between native prey or predators and non-native predators. Overall, long-term studies following initial introductions of non-native predators can improve our knowledge on evolutionary changes in native prey and predators and the decline of predator-prey and predator-predator naïveté. Our findings on body size related voracity of larger non-native lady beetle species contribute to the research on competition advantages over smaller lady beetle species, which are associated with their establishment and invasion success.

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Author contributions and status of publications

CHAPTER 2

Predation and avoidance behaviour in aphid-ladybird interactions of native and invasive ladybirds in Europe

Denise Bertleff, Jette Diekmann, Sophia Brand, Ayse Gül Ünlü, Roman Bucher

RB and AGÜ conceived and designed the study. DB, JD, SB collected the animals and conducted the experiments. DB analyzed the data and wrote the manuscript. RB and AGÜ provided statistical and editorial advice.

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CHAPTER 3

Comparison of native and non-native predator consumption rates and prey avoidance behavior in North America and Europe

Ayse Gül Ünlü, John J. Obrycki and Roman Bucher

RB and AGÜ conceived and designed the study. AGÜ and JJO collected the animals. AGÜ conducted the experiments. AGÜ analyzed the data and wrote the manuscript. RB and JJO provided statistical and editorial advice.

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CHAPTER 4

Predation and avoidance behavior of the pea aphid *Acyrthosiphon pisum* confronted with native and invasive lady beetles in Europe

Ayse Gül Ünlü, Jördis F. Terlau and Roman Bucher

RB and AGÜ conceived and designed the study. AGÜ, JFT collected the animals and conducted the experiments. AGÜ analyzed the data and wrote the manuscript. RB provided statistical and editorial advice.

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CHAPTER 5

Interactions of ants with native and invasive ladybeetles and the role of chemical cues in intraguild interference

Roman Bucher, Laura M. Japke, Ayse Gül Ünlü, Florian Menzel

RB and FM conceived and designed the study. LMJ and AGÜ collected the animals and conducted the experiments. AGÜ collected the chemical cues. AGÜ and FM identified the chemical cues. RB analyzed the data and wrote the manuscript. FM provided statistical advice. FM, LMJ, AGÜ provided editorial advice.

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CHAPTER 6

Intraguild interactions between ants and native versus non-native lady beetles in North America and the role of chemical cues

Ayse Gül Ünlü, John J. Obrycki, Florian Menzel and Roman Bucher

RB and FM conceived and designed the study. AGÜ and JJO collected the animals. AGÜ conducted the experiments. AGÜ collected the chemical cues. AGÜ and FM identified the chemical cues. AGÜ analyzed the data and wrote the manuscript. RB, FM and JJO provided statistical and editorial advice.

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Curriculum vitae

Die Seite 165 (Lebenslauf) enthält persönliche Daten. Sie ist deshalb nicht Bestandteil der Online-Veröffentlichung.

Erklärung

Hiermit versichere ich, dass ich meine Dissertation mit dem Titel

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