LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

AN ANALYSIS OF COMPETITIVE TRAITS IN PEST ANT SPECIES

Dissertation

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ABSTRACT

The successful spread of invasive species can often be explained by specific behavioral, morphological, chemical and genetic traits. Studies suggest that those traits are also present in native species that expand strikingly fast and turn into an issue for the environment. Mass occurrences of the native pest ant species *Formica fuscocinerea* have recently become a concern for leisure areas in Southern Germany. This thesis investigates whether these mass occurrences can similarly be explained by traits known from invasive species, such as a high interspecific dominance and extensive colony networks. As cooperation among large numbers of individuals requires pronounced communication abilities, this thesis also investigates whether pheromone communication contributes to the superiority of invasive ants. Therefore, competitive strength and pheromone communication of the invasive garden ant *Lasius neglectus* is compared with those of the two closely related native sister species *Lasius niger* and *Lasius platythorax*. Identifying the pheromones used for communication can facilitate more specific control of pest ant species. Targeted control methods use baits or traps that are equipped with species-specific pheromone attractants. Ants naturally use pheromone attractants produced in pheromone glands for foraging. This thesis compares hindgut, poison gland and Dufour's gland pheromones of *L. neglectus* against those of *L. niger* and *L. platythorax* to identify species-specific attractants for the invasive garden ant. The results show that the native pest ant species *F. fuscocinerea* is able to dominate other ant species by pronounced interspecific aggression. In contrast, *F. fuscocinerea* does not show intraspecific aggression among individuals from distant populations indicating weak or nonexistent colony boundaries. Thus, the striking mass occurrences of *F. fuscocinerea* can be attributed to traits known from invasive ant species. The trail communication of the invasive garden ant *L. neglectus* seems to be adapted to the exploitation of stable and productive food sources. *Lasius neglectus* shows a higher precision in following hindgut trails than the native *Lasius* species. The pheromone blends of the studied glands are notably different. Of 60 identified substances are 9 specific to the invasive *L. neglectus*, 26 to *L. niger* and 4 to *L. platythorax*. The chemical attractant 2,6-dimethyl-3-ethyl-5-hepten-1-ol can unambiguously be assigned to the hindgut of the invasive garden ant *L. neglectus*. Thus, this substance is a promising candidate for a species-specific attractant in the control of the invasive garden ant *L. neglectus*. High interspecific aggression and supercolonial structures are important traits of invasive ant species and this dissertation suggests that they likewise enable the native pest ant *F. fuscocinerea* to become dominant. A considerably more sophisticated pheromone communication does not necessarily belong to traits of invasive ants, particularly *L. neglectus*. However, the findings are provisional and require further investigation. Yet, the analyses of the communication pheromones provide a basis for the species-specific control of *L. neglectus*.

ZUSAMMENFASSUNG

Die erfolgreiche Ausbreitung invasiver Arten kann häufig mit bestimmten Verhaltensweisen, morphologischen, chemischen und genetischen Eigenschaften erklärt werden. Untersuchungen lassen vermuten, dass diese Eigenschaften auch bei den heimischen Arten vorkommen, die sich auffallend schnell ausbreiten und zu einem Problem für die Umwelt werden. Massenvorkommen der heimischen Pestameisenart *Formica fuscocinerea* wurden jüngst zu einem großen Problem auf Freizeitflächen in Süddeutschland. Diese Arbeit untersucht, inwiefern diese Massenvorkommen auf ähnliche Weise durch Eigenschaften erklärt werden können, wie sie von invasiven Arten bekannt sind, wie etwa eine hohe zwischenartliche Dominanz und ausgedehnte Kolonievernetzung. Da die Kooperation einer großen Anzahl von Individuen ausgeprägte Kommunikationsfähigkeiten benötigt, untersucht diese Arbeit zudem, ob die Pheromonkommunikation zur Überlegenheit invasiver Arten beiträgt. Dafür werden die Konkurrenzstärke und die Pheromonkommunikation der invasiven Gartenameise *Lasius neglectus* mit denen zweier nah verwandter heimischer Schwesternarten *Lasius niger* und *Lasius platythorax* verglichen. Eine Identifikation der Pheromone, die für die Kommunikation verwendet werden, kann eine spezifischere Bekämpfung von Pestameisenarten ermöglichen. Zielgerichtete Kontrollmethoden verwenden Köder oder Fallen, die mit artspezifischen Pheromonlockstoffen ausgestattet sind. Ameisen verwenden Pheromonlockstoffe, die in Pheromondrüsen produziert werden, naturgemäß bei der Futtersuche. Diese Arbeit vergleicht Pheromone aus dem Enddarm, der Giftdrüse und der Dufourdrüse von *L. neglectus* mit denen von *L. niger* and *L. platythorax* um artspezifische Lockstoffe für die invasive Gartenarmeise zu identifizieren. Die Ergebnisse zeigen, dass die heimische Pestameisenart *F. fuscocinerea* in der Lage ist, andere Ameisen durch ausgeprägte zwischenartliche Aggression zu dominieren. Im Gegensatz dazu zeigt *F. fuscocinerea* keine innerartliche Aggression zwischen Individuen von entfernten Populationen, was auf schwache oder nicht vorhandene Koloniegrenzen hinweist. Folglich können die auffälligen Massenauftreten von *F. fuscocinerea* Eigenschaften zugeschrieben werden, die von invasive Ameisenarten bekannt sind. Die Spurkommunikation der invasiven Gartenameise *L. neglectus* scheint an die Ausbeutung stabiler und ergiebiger Nahrungsquellen angepasst zu sein. *Lasius neglectus* zeigt eine höhere Präzision beim Verfolgen von Enddarmspuren als die heimischen *Lasius* Arten. Die Pheromonzusammensetzungen der untersuchten Drüsen sind deutlich unterschiedlich. Von 60 identifizierten Substanzen sind 9 spezifisch für die invasive *L. neglectus*, 26 für *L. niger* und 4 für *L. platythorax*. Der chemische Lockstoff 2,6-Dimethyl-3-ethyl-5-hepten-1-ol kann eindeutig dem Enddarm der invasive Gartenameise *L. neglectus* zugeordnet werden. Diese Substanz ist somit ein vielversprechender Kandidat für einen artspezifischen Lockstoff zur Bekämpfung der invasiven Gartenameise *L. neglectus*. Hohe zwischenartliche Aggression und superkoloniale Strukturen sind wichtige Merkmale invasiver Ameisenarten und diese Arbeit weist darauf hin, dass sie in gleicher Weise der heimischen Pestart *F. fuscocinerea* ermöglichen dominant zu werden. Eine deutlich raffiniertere Pheromonkommunikation gehört allerdings nicht notwendigerweise zu den Merkmalen invasiver Ameisen, insbesondere nicht zu denen von *L. neglectus*. Die Erkenntnisse gelten jedoch nur vorläufig und benötigen weitere Untersuchungen. Dennoch bietet die Analyse der Kommunikationspheromone eine Grundlage für die artspezifische Kontrolle von *L. neglectus*.

PRE-PUBLISHED MATERIAL

The following sections of this thesis are based on the publication:

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When you are a bear of very little brain, and you think of things, you find sometimes that a thing which seemed very thingish inside you is quite different when it gets out into the open and has other people looking at it.

— A. A. Milne, The-House-at-Pooh-Corner

CONTENTS

CHAPTER 1

AN INTRODUCTION TO INVASION BIOLOGY

Ever since humans migrated, they inevitably carried plants, seeds, spores, eggs or animals (di Castri, 1989; McNeely, 2001; Davis, 2009) mainly for food supply. At the beginning of the Holocene, humans started to settle down to specifically grow and cultivate crops and fruits on nutritious grounds, to domesticate animals (Bocquet-Appel, 2011) and to trade their goods with other regions in the world (McNeely, 2001; Mack et al., 2000). Thus, although humans are not exclusively responsible for biological invasions, intentionally or accidentally, human activities support overcoming natural barriers and subsequently species spreading in regions far apart from their native range (Mack et al., 2000).

Between the 15th and the 18th century, the age of the great discoveries, extensive overseas exploration emerged, and Europe connected to far distant regions in the world (McNeely, 2001; Mack et al., 2000). New trade routes were opened, new species and other resources were discovered and found to be suitable for utilization and trading, and global commerce rapidly increased (Mack et al., 2000). Given that numerous exports of species from the Old World, e.g. the European rabbit *Oryctolagus cuniculus* to New Zealand or Australia, or imports of species to the Old World, e.g. the raccoon *Procyon lotor*, took place after that time, biological invasions are seen as a predominantly post-Columbian phenomenon (Mack et al., 2000).

Numerous species were deliberately introduced for particular purposes: Birds, e.g. the common pheasant *Phasianus colchicus*, were imported from the East and released in the USA and Europe for hunting, fish, e.g. the rainbow trout *Oncorhynchus mykiss*, was imported for commercial fishing or for aquaristic purposes, the mink *Neovison vison* and other fur-bearing animals were imported to Europe for fur production, and plant and tree species, e.g. the potatoe *Solanum tuberosum* or the maritime pine *Pinus pinaster*, were imported for agriculture, horticulture and forestry. Furthermore, in the 19th century, colonialism led to the founding of so-called *acclimatization societies* with the purpose to enrich the local fauna with familiar animals and plants. Particularly in Australia and New Zealand the local fauna was considered to be uncivilized and deficient, so that European species were imported to make the settlers feel more comfortable and at home (Bennett, 1862).

Nowadays national and international traffic of goods has reached higher levels than ever before. A vast number of containers full of seeds, fruits, or animals are shipped around the world every day and safely reach their place of destination in short times. Most trade items have rather less direct biological impact on the ecosystems in regions they are imported to. Nevertheless, cargo containers do not always contain only the goods intended for trading. Little hitchhikers, vermin,

rats, mice or insects as well as fungi, bacteria, viruses and other pathogens join the travels (Davis, 2009). For example, the annual outbreak of the flu with its origin in East and Southeast Asia is closely connected with human travel routes (Russell et al., 2008). The influenza A virus (H3N2) is imported from Southeast Asia to North America, Europe and Australia and spreads from Europe and North America until it reaches South America (Davis, 2009; Russell et al., 2008).

After all, global trade and traffic has been leading to rapid spread of problematic flora and fauna with far-reaching consequences for native ecosystems. Hence, biology, introduction pathways and impacts of invasive species are of particular interest to scientists intending to prevent future spread and further damage.

1.1 The invasion process: Introduction, establishment and spread

Although the term invasion refers to "any process of colonization and establishment beyond a former range, particularly in which a species plays a conspicuous role in the recipient ecosystem" (Reise et al., 2006, p.78), the tendency of invasive species to spread rapidly may cause the regular association of invasions with undesirable impacts on health, economy or ecology (Davis, 2009). Basically, the main difference between natural range expansions and biological invasions is the time species need to spread over long distances (Richardson et al., 2000): Natural range expansions are usually long-lasting processes and often decelerate at geographic barriers like waters, mountains, or deserts. In contrast, invasive species or their propagules often spread by means of dispersal vectors which overcome obstacles such as geographic barriers more easily and in short time. Dust storms for instance, were found to be very effective in transporting pollen, spores, bacteria and other pathogens over thousands of kilometers even across the Atlantic Ocean (Davis, 2009; Hara and Zhang, 2012; Kellogg and Griffin, 2006). Other dispersal vectors are animals that carry and thereby disperse smaller organisms. The long-distance dispersal of larger organisms, however, is more dependent on humans (Davis, 2009). Although there are examples of larger organisms that naturally dispersed globally without the involvement of humans, they are considered to be less likely (Wyatt and Carlton, 2002).

The introduction of one or more living organisms does not automatically entail biological invasions (Williamson, 1996). The likelihood of a successful establishment and spread of a species depends on numerous factors (Carrete et al., 2012; Chapple et al., 2012). The first condition is to survive the introduction process. For this, quality and quantity of the introduced propagules are of crucial importance (Davis, 2009). Frequencies of introduction events and the number of introduced propagules (collectively the propagule pressure) represent further key factors. Numerous studies imply the importance of propagule pressure as a factor mediating successful establishment in a range of taxa, including mammals, birds, fish, invertebrates and plants (Colautti et al., 2006b; Simberloff, 2009; Johnston et al., 2009; Lockwood et al., 2005). High propagule pressure is often supported by stable transportation routes in human ware traffic (Keller et al., 2011b). Indeed, a positive correlation of the volume of international trade and establishment rates of introduced species was found for the United States (Levine and D'Antonio, 2003).

Despite high propagule pressures, some ecosystems seem to be more resistant against intruders than others. The resistance, i.e. the ease with which intruders can establish, defines the invasibility of an ecosystem and its community (Levine and D'Antonio, 1999; di Castri, 1989). Factors governing invasibility are subjects of hot debate (Levine and D'Antonio, 1999; Davis, 2009). The *diversity-invasibility hypothesis*, for example, states that environments with a higher species-richness should also have a higher resistance against invasions (Elton, 1958). Establishments of new organisms are prevented by fewer empty niches and, accordingly, by fewer available resources (Davis, 2009). However, it seems that the effect of native species diversity on influencing invasions is weak compared to the factors controlling native diversity, such as nutrient availability, habitat disturbance and species composition (Davis, 2009; Levine and D'Antonio, 1999). This is most likely due to native

and introduced species responding to the same environmental factors (Levine and D'Antonio, 1999). Disturbance events can increase resource availability, either by freeing up existing resources when disturbance-sensitive species vanish or by supplying additional resources (Davis, 2009). When the increase of resource availability occurs together with introduction events the resources can be used by the introduced propagules to become established (Davis et al., 2000).

The underlying idea of the *enemy-release hypothesis* is that newly introduced species have an advantage over native species as they leave their specialist enemies behind (Williamson, 1996; Keane and Crawley, 2002). In contrast, native species have to defend both their specialist and generalist enemies. The assumption is that intruders are attacked by native generalist enemies lesser or at similar rates compared to native species (Davis, 2009). However, the importance of the mechanisms postulated by the enemy-release hypothesis is still debated (Davis, 2009). Opponents argue that enemies with broad host ranges should easily be able to include the newly introduced species into their diet (Parker and Gilbert, 2007). Furthermore, numerous enemies are cosmopolitan, and introduced species may often be accompanied by their native enemies, e.g. pathogens (Parker and Gilbert, 2007). At the same time introduced species may actually have a disadvantage over native species as they lack the appropriate defense against native predators (Davis, 2009). Proponents, however, argue that introducing their own enemies can also be beneficial for introduced species as these non-native enemies likewise affect a less resistant environment (Davis, 2009). There are examples where the lack of natural competitors and predators favored the establishment of introduced species, particularly on isolated islands. One example is the short-tailed weasel *Mustela erminea*, which was once introduced in New Zealand to control the introduced rabbit *O. cuniculus*, and now poses a serious threat to kiwis and other hole-nesting forest birds. Similarly, the house cat *Felis catus* was accepted as a pet and biological control for introduced rats (black rat *Rattus rattus*, brown rat *Rattus norvegicus*, long-haired rat *Rattus villosissimus*) and mice (house mouse *Mus musculus*) in Australia for decades (Denny and Dickman, 2010; Abbott, 2008). Since 1980, house cats have officially been deemed as a key predator that impact many native species (Denny and Dickman, 2010).

After successfully surviving the introduction event and successfully overcoming the challenges of becoming established in the new environment, introduced species can take the next steps of reproducing and spreading. The success of these steps often depends on the presence of conspecifics. The initial population size is often the limiting factor for a successful invasion (Keitt et al., 2001). Lower species densities decrease the chance to find mating partners and to prevent inbreeding, a process that is called *the Allee-effect* (Courchamp et al., 1999). Furthermore, the higher the initial genetic diversity, the more likely is the presence of pre-adapted individuals, and the more likely are adaptations to the new environment following the introduction phase (Carrete et al., 2012; Chapple et al., 2012; Allendorf and Luikart, 2007). Unlike introduction, the subsequent spreading is often independent of humans as dispersal vectors and more comparable to natural range expansions (Davis, 2009). Nevertheless, range expansions of introduced species are often tightly linked to human-induced habitat alterations. Non-native freshwater fish species, for example, have continuously invaded and spread in Germany since the opening of the Rhine-Main-Danube canal in 1992 (Wolter and Röhr, 2010). Invasive plant species spread using road networks as disturbance corridors: The invasive smooth bedstraw *Galium mollugo* succesfully invaded Bic National Park (Quebec, Canada) along the roadsides (Meunier and Lavoie, 2012).

Urbanized areas are at particular risk of getting invaded by non-native species as they offer particularly favorable conditions for successful invasions: Firstly, urbanized areas are the places of destination of human ware traffic. Thus, the probability of introducing non-native species is high. Secondly, urbanized areas are usually disturbed habitats which can offer free resources to more tolerant newcomers. Thirdly, species populations and compositions in urbanized areas are greatly influenced by humans. Chances are that humans also influence predator-prey relationships in favor of introduced species. Last but not least, as urbanized areas are also departure points of human ware traffic and as they often additionally offer disturbance corridors to further habitats urbanized

areas facilitate further spread of already established introduced species.

1.2 Why are some species more successful invaders compared to others?

Understanding the life history traits of invasive species is necessary to control and, at best, to prevent introductions and also to identify potentially new invasive species. Several approaches have been developed to study the role of phenotypic traits as predictions of successful biological invasions.

The *target-area approach* compiles and assesses the traits of invasive species in a region and searches for commonalities explaining their successful invasion histories (Mack, 1996; Hamilton et al., 2005). The benefits of having a list of hypothesized traits that affect invasiveness are obvious as new or potential immigrants could be used for empirical tests. Traits that contribute to invasion success in weeds include, among others a wide environmental tolerance, rapid growth, pollination through unspecialized visitors or wind, continuous and high overall seed production, great longevity of seeds, lack of special requirement for germination, adaptations for short and long distance dispersals, and special means for interspecific competition (Baker, 1974). Various highly successful invaders, such as the shepherd's-purse *Capsella bursa-pastoris*, the goosegrass *Eleusine indica*, the common purslane *Portulaca oleracea* and the cogongrass *Imperata cylindrica*, possess (some of) these traits (Mack, 1996). However, there are also invasive plant species that lack almost all of these traits, while various plant species that posses those traits are nevertheless not invasive (Mack, 1996).

The *source-area approach* investigates traits of invasive species that differ from related non-invasive species of the same native source area. This approach allows the identification of traits valuable for passing the introduction and establishment phase and becoming invasive in new areas. However, the size of the native geographic range needs to be taken into account, as species with a wide distribution are more likely to be picked up and moved to new locations. Importantly, species with a wide environmental tolerance are typically more likely to succeed in a new environment (Goodwin et al., 1999). This seems to be particularly the case in plant species. Although distinct differences between invasive and non-invasive species can be identified, researchers have difficulties in reliably predicting invasiveness based on biological attributes (Goodwin et al., 1999). In contrast, the size of the geographic native range does seem to represent a reliable predictor for invasiveness (Goodwin et al., 1999).

Finally, the *native-comparison approach* compares the life-history traits of native and non-native species in the invaded area (Hamilton et al., 2005; Crawley et al., 1996; Cadotte and Lovett-Doust, 2001; Lake and Leishman, 2004). This approach identifies traits that allow the invasive species to outperform native competitors. Many studies have quantified the extent to which invasive and non-invasive species differ in their main traits (Ordonez et al., 2010). Nevertheless, it turned out that drawing generally valid conclusions from these studies is difficult. For example, a meta-analysis of 4473 plant species from over 95 communities (3784 species were measured in their native range, 689 species in their introduced range and 207 in both ranges) found only two possible (related) hypotheses affirmed for promoting successful establishment (Ordonez et al., 2010): (1) The idea of limiting similarity, states that competition with dominant native species with similar traits aggravates establishment of an invasive species (Hutchinson, 1959; Macarthur and Levins, 1967; Abrams, 1983; Van Kleunen et al., 2010), and (2) Darwin's naturalization hypothesis, states that competition with congenerics aggravates establishment of an invasive species (Daehler et al., 2001; Duncan and Williams, 2002; Strauss et al., 2006; Diez et al., 2008). This means that the more the introduced species differs from the native species community the more likely can the introduced species successfully establish in this community. This is in line with the classic *empty niches* idea to explain invasions (Ordonez et al., 2010).

In general, each approach taken in isolation has limitations, and attempts to make reliable predictions about future invasions have consequently met with limited success. Including different approaches might help to gain a better understanding of biological invasions.

1.3 Invasive ant species

Ants are among the most successful organisms on earth. The taxonomic family *Formicidae* is estimated to comprise about 20,000 species (Hölldobler and Wilson, 1990). A high biodiversity allows ants to colonize almost all terrestrial ecosystems[1] and a great variety of ecological niches (Wilson and Taylor, 1967). The adaptability of masses of highly cooperating individuals makes some ant species also the most destructive organisms on earth. Five of the 100 world most invasive organisms (a list including animals, plants and microorganisms) are ant species: The yellow crazy ant *Anoplolepis gracilipes*, the Argentine ant *Linepithema humile*, the African big-headed ant *Pheidole megacephala*, the red imported fire ant *Solenopsis invicta* and the little fire ant *Wasmannia auropunctata* (ISSG[2]).

Invasive ants spread via different pathways. Many of them are associated with human trading and construction activities such as road construction and landscaping. Due to their small body sizes ants are often transported unintentionally in cargo containing timber, soil or plants, machinery, and road vehicles (Chong and Lee, 2010; Chong and Lee, 2009). Greenhouses of botanical gardens and market gardens serve as bridgeheads for tropical species in temperate regions. In 1999, 147 ant species have been located outside their native range, though not all of them became invasive (McGlynn, 1999). Some species are now so widely spread that their native range can no longer be determined with certainty. However, native or presumed native ranges of the most invasive ant species are tropical and subtropical regions of Africa, South America and Asia (Tab 1.1). Accordingly, these species tend to invade mainly tropical, subtropical and Mediterranean regions of the world, but they also infest heated buildings like hospitals, canteens and greenhouses in temperate regions (Tab 1.1). Once introduced and established, invasive ant species cause considerable damage to the native flora and fauna, economic growth and human health.

A striking example for the impact of invasive species on the environment is the yellow crazy ant *A. gracilipes*. The native range of this species is unknown, although it is speculated that the species stems from Southern India, Sri Lanka or Southeast Asia (Wetterer, 2005, Tab 1.1). However, it is known that the yellow crazy ant was accidentally introduced on the Christmas Islands between 1915 and 1934 (Wetterer, 2005; O'Dowd et al., 1999). After decades of low and inconspicuous population densities, the species suddenly began to spread with an average spreading speed of one kilometer per year at the end of the 20th century (O'Dowd et al., 2003; O'Dowd et al., 1999). In 2001, populations already inhabited one-quarter of the island's rain forest (O'Dowd et al., 2003). Supercolonies of thousands of ants (2254 foraging ants per square meter according to Abbott, 2005) finally contributed to a rapid and catastrophic shift in the rain forest ecosystem, a so-called *invasional meltdown* (Abbott, 2005; O'Dowd et al., 2003). The primary victim of the yellow crazy ant is the endemic Christmas island red crab *Gecarcoidea natalis*. The yellow crazy ant is assumed to have killed up to 15 million red crabs, which caused a reduction of one-quarter to one-third of the entire red crab population, in recent years (O'Dowd et al., 2003). Since the Christmas island red crab includes litter, fruits, flowers and seedlings of many species in its diet, it is a keystone species for forest structures and processes (O'Dowd and Lake, 1991). The direct consequence of the elimination of the crab population is, thus, the accumulation of leaf litter and a mass recruitment of seedlings in the island rain forest areas (O'Dowd et al., 1999).

[1] except Antarctica, Iceland, Greenland, Polynesia east of Tonga and a few islands in the Atlantic and Indian oceans

[2] The Invasive Species Specialist Group (ISSG) is a global network of scientific and policy experts on invasive species, organized under the auspices of the Species Survival Commission (SSC) of the International Union for Conservation of Nature (IUCN). (http://www.issg.org; accessed August 2017)

Table 1.1: The most widespread, abundant and damaging invasive ant species in the world

Species	Subfamily[1]	Native range	Introduced range
Anoplolepis gracilipes Yellow crazy ant	F	Unknown but possibly Afrika or Asia[2,3]	Tropical Asia and tropical Island of the Indian and Pacific Oceans (not found above 1200 m elevation), tropical Africa (Dar es Salaam, Zanzibar), tropical Australia (moist monsoon rain forests), Neotropics (western Mexico), subtropical Asia (up to 26 – 27° N in northern India, southern China, and southern islands of Japan).[3]
Lasius neglectus Invasive garden ant	F	Turkey, Iran, the Black Sea area and other areas of Asia Minor[4]	Europe (including Belgium, Bulgaria, France, Georgia, Germany, Greece, Hungary, Iran, Italy, Kyrgystan, Netherlands, Poland, Romania, Spain, Switzerland and United Kingdom, Uzbekistan), Russia[5]
Linepithema humile Argentine ant	D	Parana River drainage area of subtropical Argentina, Brazil, Paraguay, and Uruguay[6,7]	Worldwide on six continents and many oceanic islands: subtropics (Mediterranean-like climates) in California, the Mediterranean, southern Africa, Australia, New Zealand, and Japan; in tropical latitudes only at higher elevations; in temperate areas as an indoor pest.[7]
Monomorium destructor Singapore ant	M	North Africa, Middle East, South Asia[8]	Worldwide: disturbed arid and semi-arid habitats in the tropics and subtropics[8]
Monomorium floricola	M	Unknown but possibly tropical Asia[9]	Worldwide in the tropics and subtropics; up to Alaska, Montreal (above latitudes of 35° indoor records)[9]
Monomorium pharaonis Pharaoh ant	M	Unknown but possibly tropical Asia[10]	Worldwide[10]
Myrmica rubra European fire ant	M	From Ireland and Portugal to central Asia and eastern Siberia and from 39° – 70° N in latitude[11]	Temperate North America[11]
Pachycondyla chinensis Asian needle ant	P	Temperate zones from Far Eastern Asia to Southeast Asia[12]	United States: along the Eastern North American coast, from Connecticut to the northernmost part of Florida[13]
Paratrechina longicornis Crazy ant	F	Unknown but possibly Southeast Asia and Melanesia[14]	Worldwide in the tropics and subtropics; indoor pest in temperate regions[14]
Pheidole megacephala African big-headed ant	M	Africa, possibly Madagascar[15]	Worldwide in tropical lowland and more temperate regions between 38.5° N and 37.8° S (indoor records in higher latitudes)[15]
Solenopsis geminata Tropical fire ant	M	New World tropics and subtropics[16]	Worldwide[16]
Solenopsis invicta Red imported fire ant	M	South America[17]	Southern North America, Caribbean, Australia, New Zealand, Taiwan, Hong Kong, Macao, China[17]
Solenopsis richteri Black imported fire ant	M	South America (Argentina, Paraguay)[18,19]	North America[20]
Tapinoma melanocephalum Ghost ant	D	Unknown but possibly Indo-Pacific[21]	Worldwide in the tropics and subtropics; indoor pest in temperate regions[21]
Technomyrmex albipes White-footed ant	D	Indo-Pacific Area[22]	United States, Australia, New Zealand, Hawaii, South Africa, Madagascar, India, China and Saudi Arabia[22,23]
Wasmannia auropunctata Little fire ant	M	Tropical Central and South America[24]	Worldwide in the tropics and subtropics[25]

[1] D = Dolichoderinae, F = Formicinae, M = Myrmicinae, P = Ponerinae; [2] Holway et al., 2002; [3] Wetterer, 2005; [4] Boase, 2014; [5] Espadaler X. and Bernal V., 2008. *Lasius neglectus* a polygynous, sometimes invasive, ant. (Available at http://www.creaf.uab.es/xeg/lasius/index.htm; accessed March 2018); [6] Tsutsui et al., 2001; Suarez et al., 2001; [7] Wetterer et al., 2009; [8] Wetterer, 2009a; [9] Wetterer, 2010a; [10] Wetterer, 2010b; [11] Wetterer and Radchenko, 2011; [12] Yashiro et al., 2010; [13] Guénard and Dunn, 2010; [14] Wetterer, 2008; [15] Wetterer, 2012; [16] Wetterer, 2011; [17] Ascunce et al., 2011; Morrison et al., 2004; [18] Wild, 2007; [19] Palomo et al., 2003; [20] deShazo et al., 2004; [21] Wetterer, 2009b; [22] Wetterer, 2002; [23] Suarez et al., 2005; [24] Foucaud et al., 2009; [25] Wetterer, 2013;

1.4 The biology of invasive ant species

Invasive ant species across all genera maintain a combination of biological traits that support their ability to both successfully colonize and take over occupied habitats by outcompeting and replacing other ant species. It is for this reason that the occurrence of the following traits in an ant species is also called the *invasive ant syndrome* (Cremer et al., 2008).

Colonies of invasive ants are polygynous, meaning that they contain multiple fertile queens that reproduce within the same colony (Hölldobler and Wilson, 1990). Polygyny occurs when virgin queens mate inside the nest or stay in the nest after mating (Hölldobler and Wilson, 1990). In contrast, a monogynous colony contains only a single fertile queen (Hölldobler and Wilson, 1990). In this case, other mated queens are not accepted and would be attacked by the queen and by the workers.

In monogynous ant species, virgin winged queens typically mate outside the nest with one or more winged males (often during nuptial flights), spread afterwards (often during dispersal flights), and found new colonies at suitable nesting sites (Hölldobler and Wilson, 1990). These single founding queens also raise their first brood of workers without help (Hölldobler and Wilson, 1990). Invasive ant queens and males waive nuptial and dispersal flights and, as they stay in the colony they can rely on numerous workers helping to rear their offspring. Thus, in invasive ant species there is no need for functional wings or strong wing muscles, which can also serve as fat reserves in single founding queens (Seifert, 2007). Accordingly, invasive ants are often found to be smaller compared to closely-related non-invasive species (Cremer et al., 2008; Holway et al., 2002).

As soon as a polygynous colony reaches a certain size a group of workers leaves the main nest together with one or more fertile queens to colonize new nesting sites. This phenomenon is called *colony budding* (Hölldobler and Wilson, 1990). With respect to the high mortality rate of single founding queens (Hölldobler and Wilson, 1990), being supported by accompanying workers can be the crucial advantage for a young queen when inhabiting new habitats. Colony budding is one common form of colony foundation in ants also in non-invasive species (for an overview see Hölldobler and Wilson, 1990; Seifert, 2007). A polydomous colony structure occurs when a single colony inhabits more than one nest (Hölldobler and Wilson, 1990) or when two or more colonies stay in contact after budding, which leads to a network of cooperating units (Seifert, 2007). In invasive ants, polydomy can reach tremendous proportions. Some so-called *supercolonies* extend over hundreds or even thousands of kilometers and in some cases consist of billions of workers and queens (Moffett, 2012). One indicative attribute of polydomous colonies is the absence of territorial aggression between its members.

Nestmate or kin recognition is a central component of social insect societies (Hölldobler and Wilson, 1990). The ability to discriminate nestmates from non-nestmates prevents a colony from getting parasitized and socially exploited (von Beeren et al., 2011). Nests and territories are vehemently defended and non-nestmates are aggressively attacked. Nestmates recognize each other by their specific colony odor, a cocktail consisting of different cuticular hydrocarbons (Howard and Blomquist, 2004). This odor varies due to genetic and environmental factors (Hölldobler and Wilson, 1990). A trait of invasive ants is reduced nestmate discrimination represented by reduced intraspecific aggression, even among physically separated nests in different environments (Suarez et al., 2002; Holway, 1998b). Accordingly, only minor differences exist, e.g. in the chemical profiles among workers of the invasive garden ant *Lasius neglectus* (Cremer et al., 2008).

A possible explanation for a reduced intraspecific aggression is a reduced genetic variability on loci coding for cuticular hydrocarbons caused by genetic bottlenecks during introduction (Tsutsui et al., 2000) or by selection after introduction (Giraud et al., 2002; Cremer et al., 2008). Indeed, introduced populations of the invasive Argentine ant *L. humile* show a reduced genetic diversity which is associated with a lower intraspecific aggression (Tsutsui et al., 2000). In contrast, in the native range populations of *L. humile* are genetically more divers and also exhibit a pronounced intraspecific aggression (Tsutsui et al., 2000). However, the ability for kin discrimination is still

available in introduced populations of *L. humile* as two supercolonies that hardly fight at encounters can be separated in Europe (Giraud et al., 2002). The higher chemical similarity in the invasive garden ant *L. neglectus* does not seem to be due to a lower allelic richness, indicating pre-adaptations prior to introduction in this case (Cremer et al., 2008). One effect of a genetic bottleneck is a high relatedness in the introduced range that also persists between distant populations (Tsutsui et al., 2000). At the same time, relatedness within nests and colonies is lower due to polygyny in introduced populations compared to populations in the native range, which poses a problem for kin selection theory (Tsutsui et al., 2000). The loss of genetic diversity is, however, considered to be a mechanism facilitating the formation of supercolonial colony structures (Tsutsui et al., 2000).

Due to the huge number of cooperating ant nests comprising large quantities of workers and sexuals producing queens, supercolonies have the capacity for unrestricted colony growth (Markin et al., 1973; Moffett, 2012). Thus, competitive dominance in invasive ant species is sometimes merely rooted in numerical dominance (Walters and Mackay, 2005). Due to a higher number of foragers, invasive ants outperform native ant species when acquiring resources. Yet, invasive species also exhibit a pronounced interspecific aggression (Rowles and O'Dowd, 2006; Holway, 1999) that enables them to defend their resources (Drescher et al., 2011; Human and Gordon, 1999) and to take over resources occupied by other ant species (Drescher et al., 2011). Differences in body size are compensated by higher aggression rates (Cremer et al., 2006; Chong and Lee, 2010).

A further trait of invasive ant species is their high foraging efficiency. Invasive ant species are usually opportunistic foragers (Holway et al., 2002). Their diet includes carrion, prey upon small invertebrates, carbohydrate-rich plants, seeds, nectar, and honeydew secreted by aphids and scale insects (Holway et al., 2002). Although all invasive ant species are omnivorous, proportions of the different food types are species-specific (Holway et al., 2002). A broad variety of food types entails independence from specific environments, which is an advantage when being introduced into new environments with a different food supply. On the other hand, dead or living preys are unstable food sources requiring an efficient foraging method. In fact, invasive ant species appear to break the discovery-dominance trade-off (also called *exploitative-interference competition trade-off*) when competing for food resources (Holway, 1999; Lach et al., 2010). This trade-off usually allows co-existence of competing ant species in the same habitat (Fellers, 1987; Holway, 1999; Morrison, 1996; Savolainen and Vepsäläinen, 1989; Schoener, 1983): Exploitative competitors are more efficient in discovering and exploiting food sources, e.g. by faster recruitment of nestmates (Fellers, 1987; Schoener, 1983). In contrast, interference competitors displace other species at encounters. This enables the take over of already occupied food sources (Fellers, 1987). Invasive species are highly efficient at discovering and dominating food sources and are thus able to outcompete both, exploitative and interference competitors (Davidson, 1998; Holway, 1999).

1.5 Pheromone communication in ants

High foraging efficiencies also depend on appropriate foraging strategies (Witte et al., 2010). To choose the optimal foraging strategy adjusted information about, e.g., the changes of food resource qualities with time, may help and offer decisive advantages in the competition with resident ant species. In ants, collective activities are coordinated using an elaborate communication system. It is based on semiochemicals (Hölldobler and Wilson, 1990), volatile molecules, synthesized and stored in different anatomical structures, e.g. exocrine glands, inside the insect body (Attygalle and Morgan, 1984). One group of semiochemicals, pheromones, are of particular interest, because they are used for intraspecific information exchange, such as attracting mating partners, marking trails, or requesting help in enemy encounters. Semiochemicals producing structures vary greatly in distribution, form and function among ant subfamilies, ant genera, ant species and even among ant castes (Hölldobler and Wilson, 1990; Niculita et al., 2007). A selection of exocrine glands in ants is presented in the following.

The mandibular glands attached to the mandibles in the head have a wide range of functions. In some species they are the most important source of so-called *alarm pheromones*, thus, playing an important role in the ants alarm communication (Attygalle and Morgan, 1984; Hughes et al., 2001). The protective role of the mandibular gland is, however, not limited to communication: In leaf-cutting ants, mandibular gland secretions act as antimicrobial substances against alien microorganisms (Rodrigues et al., 2008). Mandibular glands are, furthermore, involved in the nestmate recognition system of some ant species (Hernández et al., 2002), and serve as source of sex pheromones in males and queens of the ant genus *Polyergus* (Topoff and Greenberg, 1988). Another cephalic gland is the postpharyngeal gland which distinguishes ants from other social insects (Billen et al., 2013). As it contains species-specific hydrocarbons similar to those on the ants cuticle surface, the postpharyngeal gland is assumed to play an important role in nestmate recognition (Bagnères and Morgan, 1991; Billen et al., 2013). The majority of thoracic glands can be found in the legs. So far, up to 20 different leg glands are described (Billen, 2009). Although they perform a variety of functions, particularly the glands located in the hindlegs are included in the production of trail pheromones of several ant genera, e.g. *Pachycondyla*, *Atta*, *Camponotus* and others (Billen, 2009). The abdomen contains further exocrine glands essential for the ants' pheromone communication. The poison (venom) gland and, particularly in the ant subfamily Dolichoderinae, the pygidial gland are important tools for predation but also important components of the alarm and of the defense system. The poison gland produces formic acid (in the subfamily Formicinae) or venom, which are neurotoxic, histolytic, or both (Hölldobler and Wilson, 1990). In the genera *Monomorium* and *Solenopsis* poison gland substances were dispensed as repellents against enemy ants and other arthropodes (Hölldobler and Wilson, 1990). In some species of the subfamilies Myrmicinae and Formicinae the poison gland contains components used for recruitment and alarm communication (Hölldobler and Wilson, 1990). In *Lasius fuliginosus*, as in most other formicine ants, the immediate resource for trail pheromones is the hindgut and the rectum (Hölldobler and Wilson, 1990; Huwyler et al., 1975; Hangartner, 1967). Hindgut and rectum are not glands per se, but parts of the digestive system. Thus, depending on the species Dufour's gland, hindgut and rectum, poison (venom) gland, Pavan's gland, pygidial gland, postpygidial gland, leg glands or various sternal glands contain pheromones used for trail communication (Billen and Morgan, 1998).

Numerous ant species use pheromone trails to attract nestmates and guide them to food sources or new nesting sites. The pheromone trail communication in ants is basically a self-reinforcing system. An ant forager that discovers a valuable food source, which cannot be handled or exploited by a single individual, marks a trail back to the colony. Recruitment pheromones stimulate nestmates to follow, which for their part mark the trail according to the food source quality they find (Reid et al., 2012). The more ants exploit the food source the higher gets the pheromone concentration on the trail and, hence, the more nestmates are recruited (von Thienen et al., 2014). Once the food source is depleted or decreased in quality, arriving foragers will return to the colony without marking the trail. Additionally, the trail pheromones evaporate over time, which leads to a decrease of the pheromone concentration on the trail and results in a reduced attraction of nestmates.

According to the volatility and instability of the trail pheromones one can distinguish between recruitment (short-lasting) and trunk trail (longer-lasting) pheromones. The genus *Lasius* is one example of ants employing long-lasting trunk trails (Oster and Wilson, 1978). This foraging method is well adapted to stable longer lasting food sources, but lacks flexibility. However, studies show that *Lasius niger* ants can regulate their pheromone release according to encounters with nestmates on the trail, called *crowding negative feedback* (Czaczkes, 2014). Other models pursue the idea of a repellent pheromone that enables ants to mark a trail as non-profitable to allow greater flexibility in trail communication (Britton et al., 1998; Robinson et al., 2008; Stickland et al., 1999); but until now only one ant species, the pharaoh ant *Monomorium pharaonis*, was found to have a repellent pheromone (Robinson et al., 2008). Many of the identified molecules that induce a significant behavioral reaction act as attractants, trail or alarm pheromones (Attygalle and Morgan, 1984).

Chemical ecologists assume that information in social insects is rather carried by pheromone

cocktails than by single pheromones (Hölldobler and Carlin, 1987; Attygalle and Morgan, 1985; Morgan, 2009; Huwyler et al., 1975). Using different pheromone blends and different pheromone concentrations allows the development of specific languages. *Lasius fuliginosus* and *Lasius flavus* have highly species-specific pheromone trails, while *Lasius niger* trails also attract *L. fuliginosus*, *L. flavus* and *Lasius emerginatus* workers (Hangartner, 1967). Thus, there are numerous ant species that developed highly species-, colony- or nest-specific languages in their pheromone trails, but there are also numerous ant species, that do not express a high specificity in their trail communication (Barlin et al., 1976; Mashaly, 2010; Hangartner, 1967; Grasso et al., 2002). A specific encryption of the trail communication may be physiologically possible or beneficial only for some ant species.

1.6 Impact and management of pest (ant) species

It is estimated that 50,000 non-native animal, plant and microbial species have been introduced in the United States up to now (Pimentel et al., 2005). Although, only a small proportion has become invasive, this proportion of species causes major problems to the environment, the economy and human health. Even in Europe 10,000 non-native species are listed in the DAISIE[3] database, according to which the number of invasive species with negative impacts sums up to more than 1300 species (including > 100 terrestrial vertebrates, > 600 terrestrial invertebrates, > 300 terrestrial plants and > 300 aquatic species) (Keller et al., 2011a; Vilà et al., 2010, DAISIE[3]).

The impacts of invasive species on environments are as diverse as the species themselves, albeit the overall adverse effects are undisputed (Keller et al., 2011a): Predation, herbivory, community disruption, disease transmission, hybridization with native species, reducing genetic variation, competition, displacement and the extinction of endemic species are only the most obvious examples of ecological effects (Keller et al., 2011a; Vilà et al., 2010).

> "Invasive alien species are a major driver of biodiversity loss. In fact, an analysis of the IUCN Red List shows that they are the second most common threat associated with species that have gone completely extinct, and are the most common threat associated with extinctions of amphibians, reptiles and mammals. [...] Invasive alien species can also lead to changes in the structure and composition of ecosystems leading to significant detrimental impacts to ecosystem services, affecting economies and human wellbeing."
>
> — *IUCN*[4]

There are estimates that invasive species have contributed to 40 % of all animal extinctions for which the cause is known in the last 400 years (CBD, 2006). After the invasive American mink *Neovison vison* spread in Europe, e.g., the European mink *Mustela lutreola* has declined and is now listed as critically endangered species on the IUCN[5] Red List of Threatened Species since 2011 (Keller et al., 2011a; IUCN, 2017[6]).

Negative impacts on ecosystems caused by invasive ant species are also diverse. An important consequence of ant invasions is the competitive displacement of native ant species (Holway et al., 2002; Hölldobler and Wilson, 1990). Especially native ant species that highly resemble the ecology of

[3] Delivering Alien Invasive Species In Europe (DAISIE) was funded by the sixth framework programme of the European Commission to get a pivotal instrument in developing a Europe-wide strategy that encompasses both the geographical scale of the problem and unites the study of different taxa in marine, freshwater and terrestrial environments. (http://www.europe-aliens.org; accessed August 2017)

[4] "The International Union for Conservation of Nature (IUCN) is a membership Union uniquely composed of both government and civil society organisations. It provides public, private and non-governmental organisations with the knowledge and tools that enable human progress, economic development and nature conservation to take place together." (https://www.iucn.org; accessed August 2017)

[5] International Union for Conservation of Nature and Natural Resources (IUCN)

[6] IUCN, 2017. The IUCN Red List of threatened species. Available at: http://www.iucnredlist.org; accessed April 2018

the invasive species but are competitively inferior have a high risk of getting affected by an invasion (Holway et al., 2002). Reports indicate that ant invasions led to a species richness reduction of 70 % in native ant species and 90 % in the total number of native arthropod individuals more particularly, and invertebrates in general (Porter and Savignano, 1990; Human and Gordon, 1997). This can have wide-ranging consequences, as many invertebrates are important elements of the network in ecosystems (Human and Gordon, 1997, also see example in Section 1.3). A negative impact of invasive ants on vertebrate populations including mammals, lizards and birds is owing to, e.g. predation, nest predation or reduction of suitability of nesting sites also commonly suggested (a literature review is given in Holway et al., 2002). Further impacts occur due to mutualistic interactions: The presence of invasive ants frequently co-occurs with a local increase in the abundance of phloem-feeding homoptera (Holway et al., 2002). Increasing numbers of phloem-feeders in turn lead to weakened host plants. However, plants are not only impaired by the phloem-feeders, but also by the displacement of native (ant) mutualists like pollinators and seed-dispersers (Holway et al., 2002; Visser et al., 1996). Besides indirect effects, invasive ants also have direct negative effects on plants due to herbivory, seed predation or soil removal around root systems in the context of nesting activities (Holway et al., 2002).

Invasive species are responsible for increasing economical damage: In 1993 annual costs caused by 79 invasive species amounted to USD 1.1 billion on average per year in the United States (Pimentel et al., 2005). Recent studies estimate the economic damage of USD 120 billion per year (Vilà et al., 2010). The damage for Canadian fisheries, agriculture and forestry caused by ten invasive species summed up to CAD 187 million (Colautti et al., 2006a). In Europe, the estimated economic impact of invasive species is between EUR 12.5 billion and EUR 20 billion per year (Kettunen et al., 2009). The costs associated to allergies induced by the pollen of the invasive ragweed *Ambrosia artemisiifolia* are between EUR 17 million and EUR 47 million per year in Germany alone (Keller et al., 2011a). However, estimations of total costs caused by invasive species are difficult: Data concerning economical losses and control measures are often available only for single species within restricted areas (Colautti et al., 2006a). Furthermore, it is virtually impossible to quantitatively assess the value of economically irrelevant damages, e.g. the displacement of any species.

The economic and ecologic scale of biodiversity loss has meanwhile reached governments. The importance of preserving endangered communities has in some areas a comparable status with the minimization of economical losses. A new chapter in international cooperation began when almost all world's governments passed the *Strategic Plan for Biodiversity 2011-2020, including Aichi Biodiversity Targets* on the Convention on Biological Diversity in Nagoya, Aichi Prefecture, Japan, in 2010. The specific role of invasive alien species is addressed in Target #9:

> "By 2020, invasive alien species and pathways are identified and prioritized, priority species are controlled or eradicated and measures are in place to manage pathways to prevent their introduction and establishment"
>
> — *Convention on Biolgical Diversity, 2010*[7]

Five years later the *2030 Agenda for Sustainable Development* re-affirmed the fundamental importance of nature for human well-being. One of its targets focused specifically on invasive alien species:

> "By 2020, introduce measures to prevent the introduction and significantly reduce the impact of invasive alien species on land and water ecosystems and control or eradicate the priority species"
>
> — *Goal 15.8, UN, 2015*[8]

[7] Convention on Biolgical Diversity, 2010. Strategic Plan for Biodiversity 2011-2020 and the Aichi Targets. Available at: https://www.cbd.int/doc/strategic-plan/2011-2020/Aichi-Targets-EN.pdf. Accessed April 2018

[8] UN, 2015. Transforming our World: The 2030 Agenda for Sustainable Development. Available at: https://sustainabledevelopment.un.org/post2015/transformingourworld. Accessed April 2018

An early detection and rapid response is commonly considered to be critical for a successful management of invasive alien species. A variety of *Early Detection Rapid Response (EDRR) Plans* operating on different scales offer blueprints for coordination and leadership of action teams[9], toolkits for prevention and management practices[10] and instructions about appropriate reactions to a specific invasive organism[11]. The successful eradication of the invasive marine alga *Caulerpa taxifolia* in California, USA, is exemplary at it shows the importance of well elaborated early detection and rapid response plans. Analyzing the case *C. taxifolia* three major components essential for an effective rapid response were designated (Anderson, 2005): "(1) biological and ecological knowledge of the invading species; (2) knowledge of the invaded site (physical, ecological, and sociological); (3) sufficient field expertise and resources for immediate action." However, only the combination of all three components will result in an effective response to a new introduction (Anderson, 2005). When *C. taxifolia* was firstly discovered at Agua Hedionda Lagoon in California on June 12th, 2000, the Southern California Caulerpa Action Team highly profited from knowledge gained during a 15-year history of spread of *C. taxifolia* in the Mediterranean Sea (Anderson, 2005). Thus, within 17 days an eradication program could be started (Anderson, 2005). The earlier an eradication program begins the less space is invaded, the less space has to be be treated and the less space has to be monitored afterwards. Monitoring plays an important role in any program aimed at reducing population numbers of invasive species: The topography of the treated area as well as weather conditions can influence the success rate of an application (Causton et al., 2005). Missed, recovered or reintroduced populations can early be discovered when monitoring surveys are conducted frequently and at the right times.

Much time and money is invested in controlling and preventing introductions of invasive species. The same holds true for the control of non-invasive pest species. Knowing about specific traits enables an optimal adjustment of treatments and monitoring surveys. In ant species, e.g. foraging distances, foraging speed, nesting sites (e.g. hypogaeic nesting), activity under climatic conditions and at different times of the day are important factors that influence the effectiveness of eradication programs (Causton et al., 2005). Actually, the specific reproductive strategy of most invasive ants, i.e. spreading by colony budding while foregoing mating flights, facilitates the success of eradication programs. Although invasive ant species overcome large distances due to human transport, their natural spread takes place in restricted areas immediately adjacent to existing populations (Causton et al., 2005). Being prepared, i.e. having the knowledge to work out an elaborate response plan, and at best already pre-approved the effectiveness of the treatment, saves time and money (Causton et al., 2005).

1.7 Research questions

Understanding the specific biology of an invasive species is a key challenge to effectively control and prevent its spread. This thesis comprises three issues: The first issue focuses on *Formica fuscocinerea*, which is a native pest species in urbanized and leisure areas in Southern Germany. Competitive abilities of *F. fuscocinerea* and co-occurring ant species are studied in a natural habitat and in controlled laboratory experiments. The second issue focuses on the pheromone communication skills of pest ant species. Although several biological traits are well known to support dominance

[9] National Invasive Species Council, 2001. Meeting the invasive species challenge: Management plan national invasive species council 2001. Available at: https://www.doi.gov/sites/doi.gov/files/migrated/invasivespecies/upload/2001-Invasive-Species-National-Management-Plan.pdf. Accessed April 2018

[10] Wittenberg R. and Cock M.J.W., 2001. Invasive alien species: A toolkit of best prevention and management practices. Available at: http://www.issg.org/pdf/publications/GISP/Guidelines_Toolkits_BestPractice/Witten-berg&Cock_2001_EN.pdf. Accessed April 2018

[11] McGlynn C., 2012: Hydrilla early detection rapid response plan for Illinois. Available at: https://www.ilwaterconference.org/uploads/5/8/3/0/58302019/1_mcglynn_hydrilla_management_ilwater_septem-ber_2012.pdf. Accessed April 2018

in invasive ant species, the role of communication skills in the invasion process is in need of further investigation. This thesis studies whether competitive dominance is also supported by a more sophisticated pheromone communication in invasive ants. Pheromone communication and competitive abilities are studied and compared among the invasive garden ant *Lasius neglectus* and two closely related native *Lasius* species. The third issue focuses on species-specific pheromones in the glands of the invasive ant *L. neglectus*.

1.7.1 Competitive advantages of the native pest ant species *Formica fuscocinerea*

This section is based on Pohl et al. (2018)

The success of control strategies against invasive species highly depends on early detection and adequate response. The assessment of the threat of invasion posed by a new immigrant is a particular challenge. As described in Section 1.4 invasive ant species are often characterized by a combination of specific traits. The existence of those traits in a certain ant species is not sufficient criterion for the invasiveness of this species. However, it is a first indication for having a potential damaging immigrant when those traits are identified in a newly established and conspicuous ant species.

An ant species that recently has been receiving much attention in Southern Germany is the European ant species *F. fuscocinerea*. Known for colonizing preferentially sandy riverbanks in the alpine and pre-alpine region (Seifert, 2007), it has turned into a pest in anthropogenic areas. *F. fuscocinerea* occupies numerous public places, particularly parks and playgrounds in and around Munich where it reaches extraordinarily high densities (up to 200 nest entrances/m^2). Eradications of *F. fuscocinerea* nests from heavily infested areas have been attempted repeatedly[12]. However, the *F. fuscocinerea* populations recovered within few weeks after treatments (personal observations in 2011 and 2012). This ability to quickly recover is known from invasive ant species (Myers et al., 1998; Souza et al., 2008; Vega and Rust, 2003). Accordingly, the mass occurrences of *F. fuscocinerea* in urbanized areas might be explained by traits similar to those of invasive ants. An alternative explanation for high worker densities of *F. fuscocinerea* can be a lack of natural competitors, particular in anthropogenic habitats. However, there are some potential competitor species, such as the black garden ant *L. niger*, that are frequently found in anthropogenic habitats (Seifert, 2007). Thus, the question arises whether *F. fuscocinerea* spreads predominantly in unoccupied habitats, or whether it also invades habitats preoccupied by other ant species. If *F. fuscocinerea* invades preoccupied habitats does it co-exist with the resident ants or does it displace them? Understanding the biology of the pest ant species *F. fuscocinerea* is crucial for the estimation of its invasion potential and for the success of future control strategies. Unlike most invasive ant species, *F. fuscocinerea* is adapted to temperate climates. Thus, less invaded regions in higher latitudes could turn into risk areas for the introduction of *F. fuscocinerea*. A clarification of the biological status of *F. fuscocinerea* is important to prevent further unnoticed spread and, consequently, irreversible damage.

In a field study a *F. fuscocinerea* population is investigated in the presence of natural competitors. Bait experiments are carried out to ascertain presence and distribution of ant species. Presence and distribution are related to biotic and abiotic parameters of the habitat. Exploitative and interference competition abilities of *F. fuscocinerea* and two co-occuring ant species from the habitat are compared in laboratory experiments. Intraspecific aggression in *F. fuscocinerea* is determined on two scales: within a field population and among distant populations. This thesis investigates the following research questions:

[12] Rathaus Umschau der Landeshauptstadt München, 27/05/2015. Avialable at https://ru.muenchen.de/pdf/2015/ru-2015-05-27.pdf; Rathaus Umschau der Landeshauptstadt München, 19/05/2017. Avialable at: https://ru.muenchen.de/pdf/2017/ru-2017-05-19.pdf. Accessed April 2018

1.1) Does *F. fuscocinerea* co-exist with other ant species in natural habitats?

1.2) How are the interspecific competitive abilities of *F. fuscocinerea* and potentially co-existing species?

1.3) What kind of colony structure does *F. fuscocinerea* have?

If *F. fuscocinerea* is able to become dominant only in anthropogenic habitats that lack natural ant competitors, one would expect co-occurrences of *F. fuscocinerea* and other ant species in more natural, undisturbed, habitats. Competitive abilities of *F. fuscocinerea* and co-occurring species would then expected to be balanced. Furthermore, *F. fuscocinerea* would be subject to a discovery-dominance trade-off that would lead to a change of ant species at food sources with time. Equal abilities in defending or taking over occupied food sources would also indicate equal competitive forces of the co-occurring ant species. Even if *F. fuscocinerea* forms extended polydomous colonies with low intraspecific aggression among ants within the population, high intraspecific aggression would be expected to occur among ants of distant populations.

In contrast, if *F. fuscocinerea* exhibits traits of invasive ant species, *F. fuscocinerea* would be expected to also dominate natural habitats containing ant competitors. Competitive dominance of *F. fuscocinerea* should then also be detectable in controlled laboratory experiments. As super-colonial structures are characteristic for the success of invasive ants they could also provide for the mass occurrence of *F. fuscocinerea*. In this case, a lower intraspecific aggression would need to be observable among ants of distant populations.

1.7.2 Competitive advantages of the invasive garden ant *Lasius neglectus*

A trait of invasive ants is their efficient foraging (Section 1.4). Colonies consisting of thousands of ants are best positioned to rapidly locate and exploit food sources and to defend them against competitors. However, particularly during early stages of establishment, invasive species usually cannot outnumber resident ant colonies (Holway and Case, 2001). Foraging success can also be achieved when communication among foragers evoke the appropriate foraging strategy (Section 1.5). Thus, having an efficient and also flexible communication could help counterbalance the disadvantage of initially small worker numbers and supply the colony in a way that facilitates fast colony growth. The question is whether invasive ants use a more differentiated pheromone communication that enables them to forage better than closely related non-invasive ant species with similar biological requirements. Flexibility in pheromone communication can, for instance, be achieved by composing trail pheromones of several glands or by varying concentrations in the pheromone blends.

For the study of competitive advantages through a more sophisticated communication in invasive ant species, two approaches are combined: the source-area approach which compares invasive with closely related non-invasive species of the same native source area and the native-comparison approach which compares invasive with non-invasive species in the invaded area (Section 1.2). This study compares the invasive garden ant *L. neglectus* with its two close non-invasive relatives, the black garden ant *L. niger* and *L. platythorax* in the invaded area. *Lasius niger* and *L. platythorax* are native and widely distributed in Europe (Section 2.1). *Lasius neglectus* is one of the few invasive ant species invading temperate regions in Europe. Although it is a young invasive species it already has high damaging impacts (Section 2.1). Its success is based on several traits in accordance with the *invasive ant syndrome* (Section 1.4). However, differences in pheromone communication might help *L. neglectus* to gain further competitive advantages over *L. niger* and *L. platythorax*.

To probe the competitive abilities of *L. neglectus*, *L. niger* and *L. platythorax* different laboratory studies including exploitative and interference competition experiments and aggression tests are conducted. Additionally, four different pheromone trail experiments are conducted to gain insights into the pheromone communication of the species. The pheromone sources for the

study of pheromone communication are selected on the basis of knowledge about the pheromone communication of the subfamily Formicinae (Section 1.5). Extracts of the pheromone sources, hindgut, Dufour's gland, poison gland and mandibular gland, are analyzed to address the following key questions:

2.1) Do the extracts contain attractive or repellent components? Are there differences among the species?

2.2) How accurately do the species follow trails made of the extracts? Are there differences among the species?

2.3) To which extent do new trails made of the extracts attract the species when presented next to established natural trails? Are there differences among the species?

2.4) How do the species react when they encounter concentrated extracts that are not offered as a trail but as a single point source next to a natural trail? Are there differences among the species?

When the invasive garden ant *L. neglectus* forages more efficiently because of a more sophisticated pheromone communication one can expect detectable differences, e.g., in the attractiveness of different pheromone trails, between invasive and native species. Also, a more precise trail-following and a higher attraction to new trails can be expected for the invasive *L. neglectus* than for the native *Lasius* species. Last but not least, reactions towards extracts when offered as point sources are expected to be more differentiated in the invasive *L. neglectus* compared to the native *Lasius* species.

1.7.3 An alternative strategy to control pest ant species

A primary tool for the control of invasive ant species is the use of poison baits (Calixto et al., 2007; Causton et al., 2005). Granular poison baits usually consist of two components: A food component, e.g. a vegetable oil coated on a defatted corn grit, to attract the species and to set off recruitment and foraging (Calixto et al., 2007), and an active component to eliminate the species sooner or later. Two kinds of active components have been proven to be efficient: Toxins that kill all castes and life stages in a colony within days (Buczkowski et al., 2014) and growth regulators that sterilize the queens and prevents immature ants from maturing (Calixto et al., 2007). Compared to insecticidal sprays granular poison baits have a lower risk of affecting non-target species, e.g. by contaminating waters and poisoning fish and aquatic invertebrates (Buczkowski et al., 2014). However, there is little known about the effects of granular poison baits on non-target native ant species (Calixto et al., 2007): The study reports on the control of the invasive red fire ant *S. invicta*. The treatment, although not specific to that invasive ant, seemed to eradicate only *S. invicta* without adverse effects on the resident ant species. Rather, it seemed that the treatment supported growth of the resident ant species. However, further investigation revealed that prior to that treatment *S. invicta* has already eliminated all native ant species such that the treatment could not affect any resident ant species. After the invasive ant was eradicated by the treatment the native ant species were able to repopulate. Thus, the study could not exclude any negative effects of the used control treatment on the resident species (Calixto et al., 2007). This emphasizes the need to improve already established control methods so as to avoid any detrimental effects on native species.

The usage of synthetic insect pheromones has long been proven to effectively control insects species-specifically (Gaston et al., 1967). Especially sex pheromones are frequently used. They contain substances to which the sensory and (or) central nervous system of the target male insects is highly adapted (Gaston et al., 1967): Synthetic sex pheromones either disrupt male orientation, such that males fail to locate females for mating (Gaston et al., 1967; Burks et al., 2010) or attract males towards pheromone-baited traps (Mullen and Dowdy, 2001; Burks et al., 2010). The use of

highly concentrated sex pheromones is a well-established method to control several *Lepidoptera* species (Ryne et al., 2007; Sieminska et al., 2009; Savoldelli and Süss, 2010). As invasive ant species usually mate inside the nest instead of showing nuptial flights (Section 1.4), mating disruption using sex-pheromones, however, would not work. In ant species pheromones are also used to, e.g. attract and recruit nestmates to valuable food sources.

The native European ant species, *L. niger* and *L. platythorax*, have a high risk of being negatively affected by further spread of the invasive garden ant *L. neglectus*. However, standard control methods against *L. neglectus* are not species-specific and likewise detrimental to the native species (Rey and Espadaler, 2004). Thus, this thesis studies the following research question:

3.1) Does the invasive garden ant *L. neglectus* have species-specific pheromone gland components that could be used for species-specific poison baits?

The findings obtained from the study of differences in the pheromone communication of the invasive garden ant *L. neglectus* and the native, *L. niger* and *L. platythorax*, form a basis for the search of species-specific pheromones. Fragments of gland extracts with highly attractive effects on the ants are analyzed, subdivided into smaller units, and subsequently tested for bioactivity and species-specificity in the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*.

CHAPTER 2

MATERIAL AND METHODS

The ant species studied in this thesis currently attract attention in Europe. *Formica fuscocinerea*, though native to Southern Germany, stands out because of mass occurrences in anthropogenic areas. *Lasius neglectus* is not native to Europe, but one of the few invasive ant species that are able to cope with temperate climates. Since its first detection in Hungary, about 30 years ago, *L. neglectus* has been rapidly spreading all over Europe. Three ant species that are native to Germany are further subjects of this study: The black garden ant *L. niger*, its sister species *L. platythorax*, and *M. ruginodis* are typical representatives of ant communities in German gardens, meadows and woodlands. For the study and comparison of the ant species observations in natural habitats, experiments under controlled laboratory conditions and chemical analyses were conducted.

2.1 Biology of the species, collection sites and laboratory husbandry

Collecting activities and experimental investigations took place between 2009 and 2012. Due to high ant numbers with tens of thousands of ants in natural ant colonies, only small fragments containing several thousand ants were collected for study purposes. *Lasius neglectus* was collected with kind permission of T. Bopp, technical director of the Botanical garden in Jena (Thüringen, Central Germany). All other study species were collected in Bavaria (Southern Germany).

2.1.1 The European pest ant species *Formica fuscocinerea* (FOREL, 1874)

The European ant species *F. fuscocinerea* is distributed in the Alps and in the pre-alpine region from 6°37'48"*E* to 16°29'24"*E* and between 200 m and 1050 m height above sea level (Seifert, 2007). It appears to be absent more than 150 km north and east of the Alps (Seifert, 2007). *Formica fuscocinerea* colonizes mainly habitats of little vegetation, e.g. sandy riverbanks, railroad embankments and roadsides in cities, where it can form extensive polydomous colonies (Seifert, 2007). *Formica fuscocinerea* sometimes builds ground nests with flat mounds (Seifert, 2007). Due to their big ommatea and velocity, the workers are excellent hunters of living prey items (Seifert, 2007). Furthermore, *F. fuscocinerea* is known to extensively exploit trophobionts[1] (Seifert, 2007). Workers, thereby, cover

[1] Trophobiosis is a form of symbiotic relationship: Ants receive honeydew from aphids and other homopterans, called trophobionts, and in return protect them against predators or unfavorable weather conditions. (Hölldobler and Wilson, 1990).

distances of at least 100 m from the nest (Seifert, 2007).

2.1.2 The invasive garden ant *Lasius neglectus* (Van Loon et al., 1990)

The introduction of the invasive garden ant *L. neglectus* to Europe was likely a result of human transport of soil and plants (Czechowska and Czechowski, 2003). Genetic, chemical and behavioral analyses of European *L. neglectus* populations show only minor differences indicating a rather recent introduction with similar origin (Ugelvig et al., 2008).

The natural range of the invasive garden ant is suggested to be Asia Minor where it co-occurs with its non-invasive sister species *Lasius turcicus* (Cremer et al., 2008; Czechowska and Czechowski, 2003). *Lasius neglectus* was first discovered, and declared as a new species, in Hungary in 1990, when a population of *L. neglectus* had invaded an entire district of Budapest (Van Loon et al., 1990; Boomsma et al., 1990). In the following years this invasive garden ant has been reported from all over Europe, i.e. Spain, Belgium, France, Italy, Greece, Germany, Poland, Romania and Bulgaria (an overview is given in Espadaler et al., 2007). In 2000, when 38 sites with populations were known in Europe and Asia, *L. neglectus* has been classified as a pest species (Seifert, 2000). Furthermore, its ecological and economical impact is claimed to be comparable to that of the Argentine ant *Linepithema humile* (Seifert, 2000). Just seven years later, in 2007, the invasive garden ant has infested 77 non-native sites in 14 countries (Espadaler et al., 2007). The current geographic distribution of *L. neglectus* extends from $1°E$ to $75°E$ and from $36°N$ to $54°N$ (Seifert, 2007; Schultz and Busch, 2009).

The invasion success of *L. neglectus* can be explained by its biology. The species shows all typical traits of the invasive ant syndrome (Section 1.4). *Lasius neglectus* is one of the smallest European species of the subgenus *Lasius sensu stricto*.[2] Its virgin queens and males mate in the colony and spread by colony budding (Cremer et al., 2008). The polygynous (Van Loon et al., 1990) and polydomous colonies show a high tendency to form enormous supercolonies (Seifert, 2007). A supercolony in Spain covers an area of 14 ha and comprises an estimated number of 112,000,000 workers and 360,000 queens (Espadaler et al., 2004). *Lasius neglectus* is an opportunistic species – yet, its intensive exploitation of aphids attracts attention (Seifert, 2007). Masses of ants and aphids cause massive damage to infested greenhouses (Seifert, 2007). In infested areas the invasive garden ant suppresses other ant species due to its strong interspecific aggression (Van Loon et al., 1990; Seifert, 2007; Cremer et al., 2006). Only few ant species, such as *Lasius fuliginosus* and *Liometopum microcephalum* can withstand *L. neglectus* (Seifert, 2007). Thus, the presence of the invasive garden ant can radically change the composition of entire arthropod assemblages (Nagy et al., 2009).

2.1.3 The black garden ant *Lasius niger* (Linnaeus, 1758)

One of the most common *Lasius* species in Europe is the black garden ant *L. niger*. Its geographical distribution ranges from Western Europe ($10°W$) to the Baikal Mountains ($105°E$) and from Finland ($66°N$) to the mediterranean zone in Southern Europe (Seifert, 2007). The black garden ant is a synanthropic ant species highly tolerant of anthropogenic impacts. It is regularly found in cities, parks, gardens, meadows and on farmland where it nests in the soil or builds mounds with densities up to 108 nests/100 m^2 (Seifert, 2007). However, *L. niger* avoids dark forests and marshland (Seifert, 2007).

Colonies of the black garden ant are monogynous with a single mated queen and up to 50,000 workers (Seifert, 2007). Although it uses almost all epigean, subterranean and arboreal food sources (Seifert, 2007) the black garden ant is mainly known for attending aphids for honeydew production.

[2] The genus *Lasius* is divided into five subgenera: *Lasius* s.str., *Cautolasius*, *Dendrolasius*, *Austrolasius* and *Chthonolasius*. Only *Lasius sensu stricto* and *Cautolasius* found new colonies independently; all other groups are temporary social parasites, i.e. mated queens enter a colony of a host ant species, get rid of the host queen and let the host workers rear their first offspring. (Seifert, 2007)

2.1.4 *Lasius platythorax* (SEIFERT, 1991), a sister species of *Lasius niger*

For a long time *L. niger* and *L. platythorax* have been assumed to belong to the same species. In 1991, *L. platythorax* has been described as a distinct species and has been separated from its sister species *L. niger* (Seifert, 1991). Just as *L. niger*, *L. platythorax* is widely distributed in Europe (Seifert, 1991). However, there is a pronounced habitat segregation between the two species: In contrast to *L. niger L. platythorax* avoids cultural habitats but can be found in woodlands, bogs and fens (Seifert, 1991). Furthermore, *L. platythorax* nests mainly in dead wood but never builds soil mounds as *L. niger* does (Seifert, 2007). *Lasius platythorax* is known to react highly aggressive when being disturbed (Seifert, 2007).

2.1.5 The common Eurasian ant species *Myrmica ruginodis* (NYLANDER, 1846)

The wide geographical distribution of *M. ruginodis* ranges from Spain to Kamchatka (Russia) and in Fennoscandia up to 71°*N* (Seifert, 2007). *Myrmica ruginodis* is a typical and often dominant wood ant species that colonizes deciduous and coniferous forests but avoids human-induced habitats such as gardens, settlements and farmland (Seifert, 2007). Colonies can be both monogynous or polygynous and also sometimes polydomous (Seifert, 2007). *Myrmica ruginodis* uses almost all epigean, subterranean and arboreal food sources and also forages up in the treetops, e.g. to exploit trophobionts (Seifert, 2007).

2.1.6 Laboratory colonies of *F. fuscocinerea*, *M. ruginodis* and *L. niger*

This section is based on Pohl et al. (2018)

In summer 2010, six colony fragments[3] of *F. fuscocinerea* were collected at the study site near Dachau (Southern Bavaria, Germany, 48°15'12"*N*, 11°29'15"*E*). Additionally, seven colony fragments were collected in and around Munich (Southern Bavaria, Germany: Planegg 48°6'17"*N*, 11°26'50"*E*; Munich North 48°11'44"*N*, 11°32'36"*E*; Munich East 48°8'33"*N*, 11°37'18"*E*; Munich South 48°6'20"*N*, 11°36'8"*E*; Munich West 48°7'13"*N*, 11°30'29"*E*) and in Murnau (Southern Bavaria, Germany 47°37'52"*N*, 11°8'58"*E*). Six colony fragments of *M. ruginodis* were collected at the study site near Dachau (Southern Bavaria, Germany, 48°15'12"*N*, 11°29'15"*E*). Five colony fragments of *L. niger* were collected in the forest near the Biological Institute of the Ludwig-Maximilians-Universität (LMU) in Martinsried (Southern Bavaria, Germany 48°6'08.8"*N*, 11°27'30.8"*E*).

Each colony fragment consisted of about 1000 ants. The colony fragments were kept in plastic boxes (23 × 34 × 25 cm) with their natural nesting substrate. To cater for air exchange, a hole was cut out of the lid and secured by a plastic mesh. The inner sides of the box walls were coated with Fluon® to prevent ant escape during the experiments. Water supply was available through a falcon tube with a water soaked paper towel. The ants were provided with honey, dead crickets and fresh water once a week. All colony fragments were kept under controlled conditions of 23 °C and 60 % air humidity.

2.1.7 Laboratory colonies of *L. neglectus*, *L. niger* and *L. platythorax*

Between summer 2009 and summer 2011, 18 colony fragments of the invasive garden ant *L. neglectus* were collected in the Botanical garden of Jena (Thüringen, Germany 50°55'50.4"*N*, 11°35'09.1"*E*). 26 colony fragments of *L. niger* and 23 of *L. platythorax* were collected in the forest near the Biological Institute of the LMU in Martinsried (Southern Bavaria, Germany 48°6'8.8"*N*, 11°27'30.8"*E*).

[3] Due to high ant numbers with tens of thousands of ants in natural ant colonies, only small fragments containing several thousand ants were collected for study purposes.

The collected colony fragments contained between 1000 and 5000 ants. The colonies were kept in tightly locked plastic boxes (23 × 34 × 25 cm) with their natural nesting substrate. To cater for air exchange, a hole was cut out of the lid and secured by a plastic mesh. The inner sides of the box walls were coated with Fluon® to prevent ant escape during the experiments. Due to the risk of keeping invasive species, the boxes housing *L. neglectus* were additionally placed on basins filled with soapy water.

For the interspecific competition experiments, nests were assembled with standardized ant numbers (13 nests with *L. neglectus*, six with *L. niger* and nine with *L. platythorax*). Each nest consisted of 1000 to 1500 ants, comparable numbers of egg clusters, larvae and pupae. Numbers were determined by weighing the ants with a laboratory scale.[4] The standardized colonies were kept in tightly locked plastic boxes (18 × 25 × 15 cm) with a 2 cm thick, moistened gypsum floor that contained cavities of 1 cm depth as nesting space. A 2 cm thick layer of the original nesting substrate was additionally dispersed on the floor.

All ant colonies were kept in a climate chamber at a constant air humidity of 65 % and a temperature of 22 °C at daytime (13 hours) and 17 °C at nighttime (11 hours). Water supply was available through a falcon tube with a water soaked paper towel. The ants were provided with honey, dead crickets and fresh water once a week.

2.2 Field studies

This section is based on Pohl et al. (2018)

To estimate the competitive dominance of *F. fuscocinerea* under natural conditions an ant community was studied in a habitat near Dachau (Southern Bavaria, Germany, 48°15'12"*N*, 11°29'15"*E*). The study area was an about 400 m long and 5 to 20 m wide soil embankment separating two lakes. Vegetation and substrate were identified and according to differences four sections within the study area were determined (Figure 2.1, Section 3.1). Observations took place between June and July 2010.

2.2.1 Bait experiment

Ant counting at baits to investigate the occurrence and abundances of ant species was conducted while considering habitat characteristics: Specimen of the vegetation and substrate were determined, as well as height of the vegetation and degree of coverage, sun exposure of the bait station, temperature at each bait station and humidity at a shady point in the habitat (measured with a thermometer and a hygrometer; Table in Figure 3.1 in Section 3.1). Thirty-five bait stations, i.e. artificial food sources containing approximately 5 g of honey and 5 g of tuna at 15 cm distances, were placed in a line every 11 m along the embankment. On five days (28th and 30th June, 2nd, 6th and 9th July) the number of ants was counted up to a maximum of 40 individuals per species at each of the baits, which was the number of ants at which a bait was fully occupied. Additionally, sun exposure (categorized as *sunny*, *semi-shady* and *shady*) and temperature at every baiting station, and the relative air humidity at one shaded point in the habitat were recorded. Counts and measurements were repeated seven times over the course of the experimental days (at 8:30 a.m., 9:30 a.m., 10:30 a.m., 3:30 p.m., 4:30 p.m., 8.30 p.m., 9:30 p.m.). The baits were regularly refilled.

The distribution of ant species in the study area was analyzed as a function of biotic and abiotic habitat parameters. A resemblance matrix was calculated from the ant numbers of each species at the baits using Bray-Curtis similarities. The matrix was analyzed with a PERMANOVA[5] (PRIMER 6 version 6.1.13 (PRIMER-E Ltd., Ivybridge, UK) extended by the PERMANOVA+ Add-In version 1.0.3) using a design with six factors (*date of observation*, *time of observation*, *section*, *bait station*, *bait type*

[4] The average weight of a single ant was calculated by weighing ten groups of ten ants and dividing the total weight by 100

[5] A short overview of the PERMANOVA is given in Appendix A.1.1

Figure 2.1: Study site near Dachau: According to substrate and vegetation characteristics four habitat sections (section 1 in blue; section 2 in yellow; section 3 in red; section 4 in green) were distinguishable on the embankment. Adapted from Pohl et al. (2018).

and *sun exposure*) and two covariates (*temperature* and *humidity*) (Table A.1 in Appendix A.1.2). The factor *time of observation* was nested in the factor *date of observation*, since the numbers of ants recorded at consecutive counts at a given observation day were considered to be dependent. The factor *bait station* was nested in the factor *section*, since each bait station was assigned to only one section. The factor *bait type* was nested in the factor *bait station*, since the numbers of ants at both bait types of a bait station were considered to be dependent. The factor *sun exposure* was also nested in the factor *section* since the sections considerably differed in vegetation cover. Statistical significances were tested using a random subset of 9999 permutations of residuals under a reduced model. The influence of the ambient air temperature on the ant numbers at the baits was evaluated using Pearson's correlation coefficient in XLSTAT (Version 2010.3.06, Addinsoft). Food preferences (carbohydrate vs. protein) were analyzed by comparing the number of ants at the two bait types (honey and tuna) with Wilcoxon signed-rank tests in XLSTAT (version 2016.01.26717, Addinsoft).

2.2.2 Foraging activity

At certain times during the day (9^{th} July at 10:00 a.m., 3:00 p.m.) and the night (18^{th} July at 11:00 p.m.; 19^{th} July at 12:00 a.m.), the numbers of *F. fuscocinerea* ants crossing a 3 cm long horizontal line on seven different spruce trunks were counted for one minute. Ants were classified as climbing up or down the trunk. The total number of ants foraging on each tree was extrapolated by using the width of the ant trail.

A resemblance matrix was calculated from the activity data, i.e. the numbers of *F. fuscocinerea* ants foraging on each tree in different directions at daytime and nighttime, using Bray-Curtis similarities. Different observation times were subsumed for the respective day or night. Data were

analyzed with a PERMANOVA (PRIMER 6 version 6.1.13 (PRIMER-E Ltd., Ivybridge, UK) extended by the PERMANOVA+ Add-In version 1.0.3) using a test design with three factors *daytime, direction* and *tree* and all possible interactions between the factors (Appendix A.1.3). Statistical significance was tested using a random subset of 9999 permutations of residuals under a reduced model.

2.3 Studying inter- and intraspecific competition

Studying inter- and intraspecific competition abilities of ant species is crucial for understanding structures and processes in ant communities. Interspecific competition explains the species composition within a community. Dominance hierarchies, niche differentiation and competitive exclusion play an important role in this respect. Intraspecific competition, in contrast, describes the colony structures and distribution within a species.

2.3.1 Exploitative and interference competition experiment (EIC)

This section is based on Pohl et al. (2018)

To compare the interspecific competitive abilities of different ants species during foraging the exploitative and interference competition (EIC) experiment, an experiment consisting of two parts, was developed. The first part tests the exploitative ability of an ant species, specifically recruitment in the absence of competitors. The second part tests the interference ability at already occupied food sources, accordingly in the presence of a competitor species.

For the EIC experiment three platforms (15 × 10 cm) connected via bridges (15 × 2 cm) were placed between two allospecific colonies (Figure 2.2). The platform in the middle was provided with a drop of honey as food source. During the first part of the EIC experiment only one species (the explorer species) was given access to the food platform. Beginning with the first ant finding the food source, the number of ants at the food source was counted in regular intervals for 14 minutes (*L. neglectus*, *L. niger* and *L. platythorax*: every 30 seconds; *F. fuscocinerea*, *M. ruginodis* and *L. niger*: every 60 seconds). After 14.5 minutes, the second part of the EIC experiment started and the second species (the competitor species) was also given access to the food source for further 15 minutes. Subsequently, the number of ants of each species at the food source was counted in regular intervals (30 seconds or 60 seconds, respectively). Food discovery time, i.e. the time that elapses between connecting the explorer colony to the platforms and the first ant arriving the food source, was additionally measured in trials with *L. neglectus*, *L. niger* and *L. platythorax*.

Resemblance matrices based on Euclidean distances were calculated for the first part (exploitative competition) from the numbers of explorer ants at the food source and for the second part (interference competition) from the numbers of competitor ants at the food source. Data were analyzed using a PERMANOVA (PRIMER 6 version 6.1.13 (PRIMER-E Ltd., Ivybridge, UK) extended by the PERMANOVA+ Add-In version 1.0.3). The test designs included the factors *explorer species* or *competitor species*, and *trial number* (with *trial number* nested in *explorer species* or *competitor species*), the covariate *time* and the interaction between *explorer species* or *competitor species* and *time*. Comparisons of interest were pre-defined as contrasts (Tables A.3 and A.5 in Appendix A.1.4). To analyze the influence of competition on the food discovery of the respective species, the ant numbers of the second part (under competition) were compared to those of the first part (without competition). The respective test design included the two factors *group* and *trial number* (with *trial number* was nested in *group*), the covariate *time*, and the interaction between *group* and *time*. Comparisons of interest were pre-defined as contrasts (Tables A.4 and A.6 in Appendix A.1.4). Statistical significances were tested using random subsets of 9999 permutations of residuals under a reduced model. To evaluate mutual exclusion of species at food sources (competitive displacement), Spearman rank correlations between the number of competitor ants and the number of explorer ants were calculated in XLSTAT (version 2010.3.06, Addinsoft). The food discovery times of *L. neglectus*,

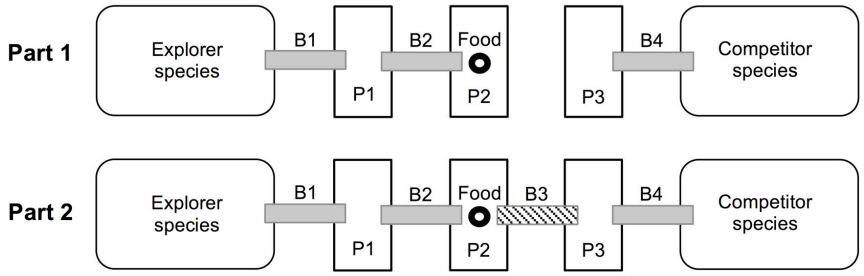


Figure 2.2: Exploitative and interference competition experiment. Three platforms (P1, P2, P3), connected via bridges (B1, B2, B3, B4), are placed between two allospecific colonies. Part 1: Only the explorer species is allowed access to the food source on P2. Part 2: The competitor species is given access to the food source. Adapted from Pohl et al. (2018).

L. niger and *L. platyhtorax* were compared using a Kruskal-Wallis test (Dunn's procedure) in XLSTAT (version 2016.01.26717, Addinsoft).

EIC with *F. fuscocinerea*, *M. ruginodis* and *L. niger* Species combinations were tested in 40 trials: For ten trials each, *F. fuscocinerea* as the explorer species had sole access to the food source in the first part and competed against *M. ruginodis* and *L. niger*, respectively, in the second part. Similarly, for ten trails each, *M. ruginodis* and *L. niger* were free of competitors in the first part and then competed against *F. fuscocinerea* in the second part. The competitive role of *F. fuscocinerea*, i.e. being the explorer or the competitor species, alternated between the trials.

EIC with *L. neglectus*, *L. niger* and *L. platythorax* In order to ensure similar conditions and to eliminate advantages solely from superior ant numbers, test colonies were standardized in ant numbers. Each test colony consisted of 1000 - 1500 ants, and clusters of eggs and larvae. Species combinations were tested in 80 trials: For 20 trials each, *L. neglectus* as the explorer species had sole access to the food source in the first part and competed against *L. niger* and *L. platythorax*, respectively, in the second part. Similarly, for 20 trails each, *L. niger* and *L. platythorax* were free of competitors in the first part and then competed against *L. neglectus* in the second part. The competitive role of *L. neglectus*, i.e. being the explorer or the competitor species, alternated between the trials.

2.3.2 Aggression experiments

Tests that quantify inter- and intraspecific aggression can indicate the ability of ants to identify ant individuals of foreign species or foreign colonies (Roulston et al., 2003). Different experimental settings were used to bring ants into encounters. The behaviors shown by the ants involved in the encounter are noted and categorized as *neutral* (e.g. moving, standing, antennating), *aggressive* (e.g. attacking, fighting, spinning), *peaceful* (e.g. grooming, feeding) or submissive (e.g. escaping) behavior. A normalized aggression index (AI) (von Beeren et al. 2012; von Beeren et al. 2011) is calculated for each trial, where

$$AI = \frac{I_a - I_{ps}}{I_t} \tag{2.1}$$

with I_a denoting the number of aggressive interactions, I_{ps} denoting the number of peaceful or submissive interactions, and I_t denoting the sum of aggressive, peaceful or submissive and neutral interactions. Hence, an aggression index of $AI = 1$ indicates entirely aggressive encounters while an aggression index of $AI = -1$ indicates entirely peaceful or avoiding encounters.

Table 2.1: List of behaviors expressed by *L. neglectus*, *L. niger* and *L. platythorax* in one-on-one encounters

Behavior	Definition	Category
attacking	Aggressive behavior towards the other ant (i.e. biting, snapping, raising the gaster)	aggressive
fighting	Aggressive behaviors of both ants, where the initial aggressor can not be identified	aggressive
spinning	Ant runs quickly in a cirle	aggressive
opening mandibles	Ant opens its mandibles while moving or standing	aggressive
grooming	Ant treats the other ant with its mouthparts in a peaceful way	peaceful
antennating	Ant touches the other ant with its antennas	neutral
moving	Ant moves in the arena	neutral
standing	Ant does not move	neutral
jerking gaster	Ant moves its gaster rapidly up and down	neutral
self-grooming	Ant treats itself with its mouthparts	neutral

2.3.2.1 One-on-one aggression test

The readiness to act aggressively can depend on the number of nestmates participating in the encounter (Tanner, 2008). Thus, an aggression test in a one-on-one situation was conducted to ensure equal chances for both parties. A single ant from one species and a single ant from another species were carefully transferred to a Petri dish after given the time to settle down in separate Petri dishes for two to five minutes. For three minutes the behaviors of both ants were recorded every 15 seconds (Table 2.1). In case of aggressive interactions, the species that started the attack was noted.

This setting was used to study interspecific aggression with *L. neglectus*, *L. niger* and *L. platythorax*. For each species combination, *L. neglectus* vs. *L. niger* and *L. neglectus* vs. *L. platythorax*, 15 trials were conducted. Additionally, six control trials were conducted for each species with both ants originating from the same colony. Aggression indices were calculated for the first twelve interactions (i.e. $I_t = 12$) of each trial and analyzed using Kruskal-Wallis tests (Dunn's procedure, Monte-Carlo P-values) in XLSTAT (version 2016.01.26717, Addinsoft).

2.3.2.2 Aggression test with groups of ants

The EIC experiment (Section 2.3.1) provides a competitive situation under more natural conditions: Usually, at first instance, only few ants of one species arrive at a food source that is already occupied by another species. Thus, over the course of the EIC experiment with *L. neglectus*, *L. niger* and *L. platythorax* an aggression study was conducted at the beginning of the second part of the experiment. The first five encounters between the competing species were recorded with a digital video camera (JVC Hybrid, Hard Disk Camcorder Everio). Only encounters next to the food source with a maximal distance of 2 cm were counted to ensure that the ants that are involved in the encounter have already discovered the bait as a valuable food source. The behaviors shown by both ants involved in the encounter were noted (Table 2.2).

Aggression indices were calculated for the first five interactions (i.e. $I_t = 5$) of each trial. The species combinations, *L. neglectus* vs. *L. niger* (LNE(LN)[6], LN(LNE)) and *L. neglectus* vs. *L. platythorax* (LNE(LP), LP(LNE)), were tested in 80 trials (Section 2.3.1). However, the number of evaluable trials varied between the species pairs as not all trials provided five distinct encounters: $N_{\mathrm{LNE(LN)}} = 15$, $N_{\mathrm{LN(LNE)}} = 10$, $N_{\mathrm{LNE(LP)}} = 15$, $N_{\mathrm{LP(LNE)}} = 9$. A resemblance matrix based on Euclidean distances was calculated for the aggression indices and analyzed using PERMANOVA post-hoc pairwise comparisons

[6]The competitor species is given in brackets.

Table 2.2: List of behaviors expressed by *L. neglectus*, *L. niger* and *L. platythorax* during the first five interspecific encounters at the beginning of the second part of the EIC experiment

Behavior	Definition	Category
attacking	Aggressive behavior towards the competitor (i.e. biting, snapping, raising the gaster)	aggressive
defending	Aggressive behavior as a response to an attack (i.e. biting, snapping, raising the gaster)	aggressive
fighting	Aggressive behaviors of both ants, where the initial aggressor can not be identified	aggressive
escaping	Avoiding behavior as response to a contact or to an attack	submissive
exploring	The intruder ant rapidly runs on the top of the competitor ants at the food source; often mandibles wide open and jerking gaster but without attacking	neutral
no reaction	No reaction after two ants come into contact	neutral

(PRIMER 6 version 6.1.13 (PRIMER-E Ltd., Ivybridge, UK) extended by the PERMANOVA+ Add-In version 1.0.3) considering the factor *species combination* (Appendix A.1.6). Statistical significances were tested using random subsets of 9999 permutations of residuals under a reduced model. For comparisons within species, i.e. the comparison of the aggression indices of a species as explorer and as competitor, Mann-Whitney U tests were conducted in XLSTAT (version 2016.01.26717, Addinsoft).

2.3.2.3 Intraspecific aggression test

This section is based on Pohl et al. (2018)

Aggressive encounters to verify colony boundaries within a species can be provoked by exposing a single ant of one colony to a group of ants of another colony (Tanner, 2008). This experimental design was used to study intraspecific competition in *F. fuscocinerea*. Ten ants from one colony (receiver ants) and a single color marked ant from a distant colony (intruder ant) were carefully transferred to a Petri dish after been given the time to settle down in separate Petri dishes for two to five minutes. The first 20 interactions (i.e. $I_t = 20$) between the receiver ants and the intruder ant were scored from video recordings (Table 2.3).

In a *small distance approach* aggression was tested among ants that came from nearby locations. Eight colony fragments were excavated every 5 to 10 m within the study area and tested in 47 different combinations (distance: 5 - 65 m, mean: 31.4 m). In eight control trials, one for each colony fragment, the effect of color marking was tested with both receiver and intruder ants originating from the same colony. A *large distance approach* was carried out to study aggression among ants from distant colonies. One colony fragment which was collected in Planegg (Southern Bavaria, Germany) served as a source for receiver ants. Further six colony fragments collected at different sites in Southern Bavaria (Germany) provided intruder ants. The distances between the receiver colony and the intruder colonies were 5 km (to Munich West), 11 km (to Munich South), 12 km (to Munich North), 13 km (to Munich East), 16 km (to Dachau) and 58 km (to Murnau). The minimum distance among the intruder colonies was 4 km. Each intruder colony was tested with the receiver colony ten times. In ten control trials the effect of color marking was tested with both receiver and intruder ants originating from the receiver colony. Additionally, the ability to recognize foreign ants and to exhibit aggressive behavior was tested in ten control trials for the *small distance approach* and in ten control trials for the *large distance approach* with *Formica* sp. as intruder.

By using the Observer software (Noldus Observer XT 9.0) various behaviors of receiver ants towards the intruder ant were recorded, counted and categorized (Table 2.3). To differentially

Table 2.3: List of behaviors shown in intraspecific encounters among *F. fuscocinerea* ants of distant colony fragments (*small distance approach*) or distant populations (*large distance approach*).

Behavior	Definition	Category
attacking	Aggressive behavior between receiver and intruder ant (i.e. biting, snapping, raising the gaster)	aggressive
grooming	A receiver ant treats the intruder ant with its mouthparts in a peaceful way	peaceful
physical contact	Receiver and intruder ant stay into physical contact without any other interaction	neutral
slow antennating	An ant slowly touches the other ant with its antennas	neutral
quick antennating	An ant quickly touches the other ant with its antennas	neutral

weight short-lasting and longer-lasting interactions ongoing interactions were re-counted every 15 seconds. Aggression indices were calculated from the data and analyzed. Additionally, numbers of fast antennation interactions were analyzed separately. Data were Spearman-rank-correlated with the distances among the colony fragments using XLSTAT (version 2016.01.26717, Addinsoft). Furthermore, data of treatment groups were compared to those of control groups using Kruskal-Wallis tests and post-hoc pairwise comparisons (Dunn's procedure, Monte-Carlo P-values) in XLSTAT (version 2016.01.26717, Addinsoft).

2.4 Studying pheromone trail communication

Pheromone trail communication was studied in the three species *L. neglectus*, *L. niger* and *L. platythorax*. The basic setting of four different experiments for the study of pheromone trail communication in ants consisted of a foraging arena containing a start and a food platform (Figure 2.3). The food platform was equipped with a drop of honey as a food source which was replenished if necessary. Depending on the experiment up to three inter-platforms were placed between the start and the food platform. All platforms were connected via replaceable cartridge bridges. The start platform was continuously connected to the test ant colony via a wooden bridge. At the beginning of each trial the start and the food platform were connected as long as the ants required to establish a stable ant trail consisting of a minimum of ten ants to the food source.

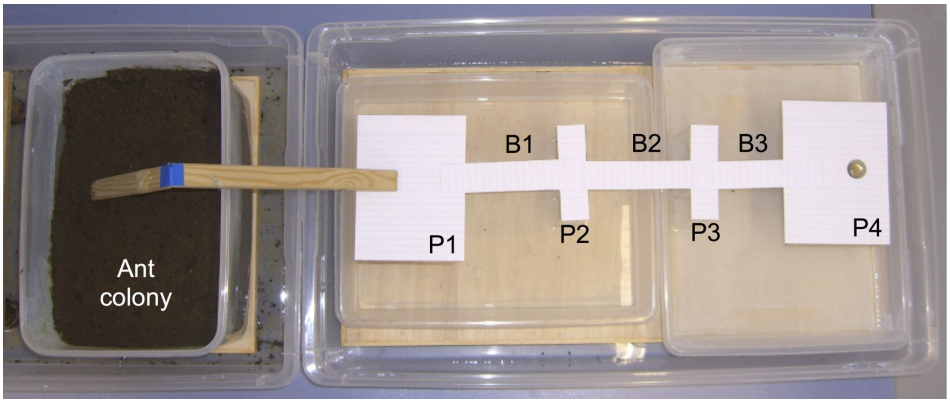


Figure 2.3: Start setting of the pheromone trail experiments. The ants have direct access to the food source on platform P4. P1: start platform; P2, P3: inter-platforms; P4: food platform; B1 - B3: cartridge bridges.

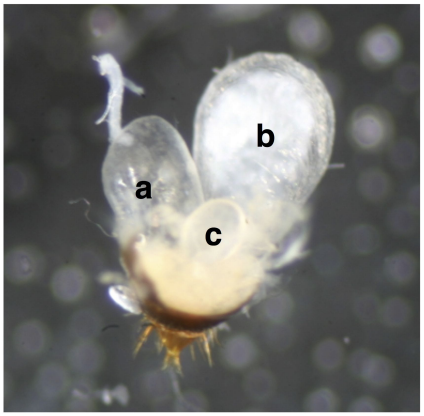


Figure 2.4: Pheromone glands in the abdomen of *Lasius* ants: Hindgut and rectum (a), poison gland (b), Dufour's gland (c)

2.4.1 Dissection of pheromone glands

For each experiment between two and nine different colony fragments per species were used for behavioral essays and, if available, one or two additional colony fragments for gland extractions. Gland substances were freshly extracted on each experimental day with species-specific glands of the respective test species. Therefore, ants were killed via freezing for several minutes. For each gland type (Dufour's gland, poison gland, hindgut[7] and mandibular gland, respectively; Figure 2.4) five equal glands were dissected in water and transferred to glass vials filled with 100 µl dichloromethane (DCM). Forceps were cleaned after each gland transfer and the dissection water was changed after each ant to avoid cross contaminations. To control for contaminations with undecane, a hydrocarbon found in extraordinary high concentrations in Dufour's glands, 1 µl of each extraction was analyzed with a coupled gas-chromatograph and mass-spectrometer (GC-MS) (description of the functioning of a GC-MS is given in Section 2.5). Only poison gland, hindgut and mandibular gland extractions with undecane contaminations of less than one percent of the undecane amount of an average Dufour's gland were accepted for testing and diluted in 1 ml DCM.

2.4.2 Direction-by-choice experiment

To test whether the ants are attracted or deterred by the content of the test glands a direction-by-choice experiment was conducted. After the start setting and a successful establishment of a stable ant trail all ants in the arena and parts of the bridges (B2 and B3) were removed and replaced by a 13.5 cm long Y-bifurcation (Figure 2.5). On one leg of the Y-bifurcation 3.33 µl/cm gland solution was applied as a solid line using a 10 µl capillary. On the other leg 3.33 µl/cm DCM was applied to control for the solvent. If one *gland equivalent* (GE) is defined as the amount of pheromones contained in a single gland then 1 cm artificial pheromone trail consisted of 0.017 GEs. After attaching the Y-bifurcation in the foraging arena the direction pursued by the first ten ants was determined. The ants had to follow the complete length of a bridge leg to get counted. Each gland type was tested in 20 trials per species. Gland type and trail direction on the Y-bifurcation alternated between trials to prevent learning effects by the ants.

Preferences for one or another leg of the Y-bifurcation within a species and a type of treatment were evaluated using Wilcoxon signed-rank tests in XLSTAT (version 2016.01.26717, Addinsoft).

[7] Hindguts and rectums were dissected together; thus, all extracts titled with hindgut also contained the substances from the rectum

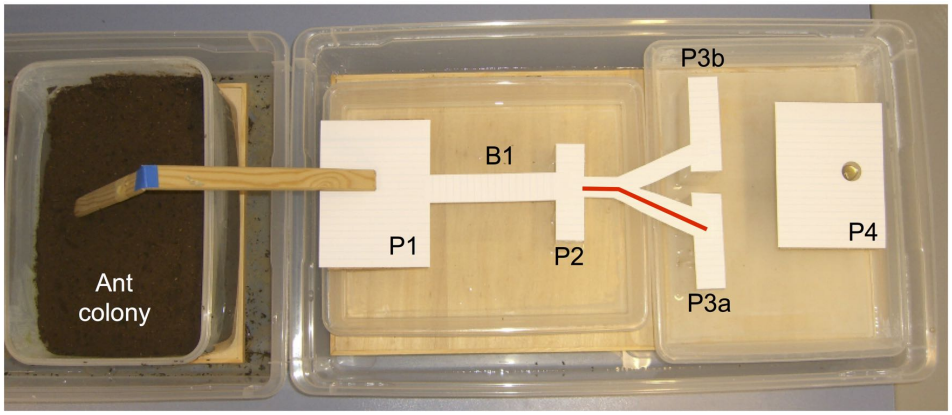


Figure 2.5: Experimental setting of the direction-by-choice experiment. The cartridge bridges B2 and B3 are replaced by a Y-bifurcation. An artificial pheromone trail (in this figure illustrated by the red line) is applied to one leg and a solvent control to the other leg of the Y-bifurcation. P1: start platform; P2, P3: inter-platforms; P4: food platform; B1: cartridge bridge.

Differences among gland types and differences among species were analyzed using PERMANOVA and PERMANOVA post-hoc pairwise comparisons (PRIMER 6 version 6.1.13 (PRIMER-E Ltd., Ivybridge, UK) extended by the PERMANOVA+ Add-In version 1.0.3). A resemblance matrix based on Bray-Curtis similarities was calculated from the data. The PERMANOVA design included the factors *species* and *gland type* and the combination of *species* and *gland type* (Table A.9 A in Appendix A.1.7.1 and Table A.16 A in Appendix A.1.7.3). To identify differences among gland types within a species the factor *gland type* was nested in the factor *species* (Table A.9 B in Appendix A.1.7.1 and Table A.16 B in Appendix A.1.7.3). To compare species within gland types the factor *species* was nested in the factor *gland type* (Table A.9 C in Appendix A.1.7.1 and Table A.16 C in Appendix A.1.7.3). Statistical significances were tested using random subsets of 9999 permutations of residuals under a reduced model.

Trail specifity in *Lasius* ants The direction-by-choice experiment was also used to test species-specifity of hindgut extractions with *L. neglectus*, *L. niger* and *L. platythorax*. Thereby one leg of the Y-bifurcation was treated with a conspecific extract and the other leg with a allospecific extract. Each trail combination was tested in 20 trials per species.

2.4.3 Accuracy-in-trail-following experiment

The accuracy with which ants follow artificial winding trails indicates which glands the ants use for foraging trails. After the successful establishment of a stable ant trail all ants in the arena and parts of the bridges were removed and replaced by a paper card (10.5 × 15 cm)(Figure 2.6). On the paper card an artificial trail was applied with 3.33 µl/cm gland solution along a 27 cm long S-shaped pencil-curve. Four gland types, DCM and the influence of the pencil-curve were tested in 20 trials each. Treatment and trail direction (clockwise or counter-clockwise) alternated between trials to avoid influences due to learning effects. After being connected with the start platform the paper card was recorded for two minutes with a video camera in an overhead shot. The numbers of ants that followed one-quarter, half, three-quarters and the entire length of the trail were counted by reviewing the video recordings.

Data were analyzed for a species using PERMANOVA and PERMANOVA post-hoc pairwise comparisons (PRIMER 6 version 6.1.13 (PRIMER-E Ltd., Ivybridge, UK) extended by the PERMANOVA+

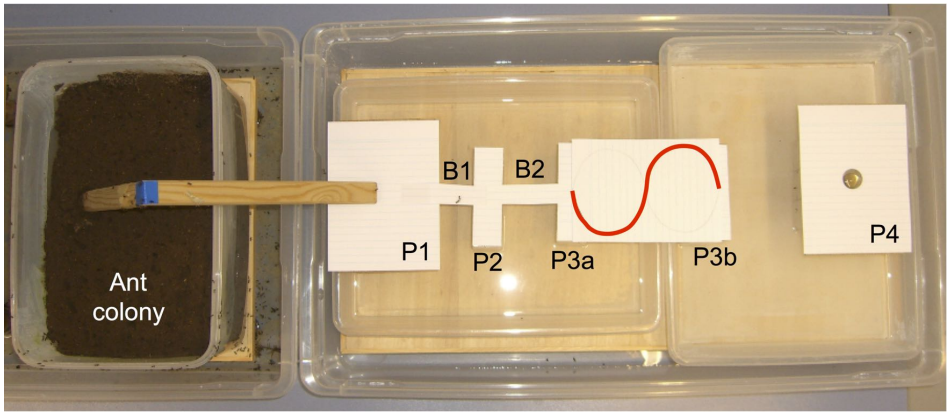


Figure 2.6: Experimental setting of the accuracy-in-trail-following experiment. The cartridge bridge B3 is replaced by a paper card. An artificial pheromone trail (in this figure illustrated by the red line) is applied along a S-shaped pencil line. P1: start platform; P2, P3a, P3b: inter-platforms; P4: food platform; B1, B2: cartridge bridges.

Add-In version 1.0.3). Resemblance matrices were calculated from the data using the Canberra metric. The PERMANOVA design took four factors into account, i.e. *test nest*, *gland type*, *trail section* and *direction* as well as the interactions between the factors (Table A.12 A in Appendix A.1.7.2). To identify differences among gland types with regard to the trail length the factor *gland type* was nested in the factor *trail section*, and *direction* was nested in *gland type* (Table A.12 B in Appendix A.1.7.2). To identify differences among trail sections within a gland type the factors *trail section* and *direction* were nested in the factor *gland type* (Table A.12 C in Appendix A.1.7.2). To identify differences among species a resemblance matrix using Canberra metric was calculated for the data of all three species. The respective PERMANOVA design included the factors *trail section*, *gland type* and *species*. The factor *gland type* was nested in *trail section* and the factor *species* was nested *gland type* (Table A.14 in Appendix A.1.7.2). As a result species were only compared within the same gland type and also only for the same trail section. Statistical significances were tested using random subsets of 9999 permutations of residuals under a reduced model.

2.4.4 Alternative-trail-branch experiment

To determine the flexibility and willingness of ants to leave trunk trails and to follow new and unfamiliar trails an alternative-trail-branch experiment was conducted. In this experiment an artificial trail branch was offered next to the natural ant trail on the connection between start and food platform that stayed untouched during the trials. Therefore, halfway between the start and the food platform an 8 cm long treated bridge branched at an angle of 90 degrees from the natural trail (Figure 2.7). The artificial trail consisted of 3.33 µl/cm gland solution or 3.33 µl/cm DCM applied as a solid line. Four gland types and a DCM control were tested in 20 trials each. Treatment and branch direction, right- or left-handed, alternated between trials to prevent learning effects by the ants. The first ten ants crossing the branch were noted whether they past or followed the branch.

Preferences for the natural trail or the branching artificial trail within a species and a type of treatment were evaluated using Wilcoxon signed-rank tests in XLSTAT (version 2016.01.26717, Addinsoft). Differences among gland types and differences among species were analyzed using PERMANOVA and PERMANOVA post-hoc pairwise comparisons (PRIMER 6 version 6.1.13 (PRIMER-E Ltd., Ivybridge, UK) extended by the PERMANOVA+ Add-In version 1.0.3). A resemblance matrix based on Euclidean distances was calculated from the data. The PERMANOVA design included the factors *species* and *gland type* and the combination of *species* and *gland type* (Table A.9 A in Appendix A.1.7.1

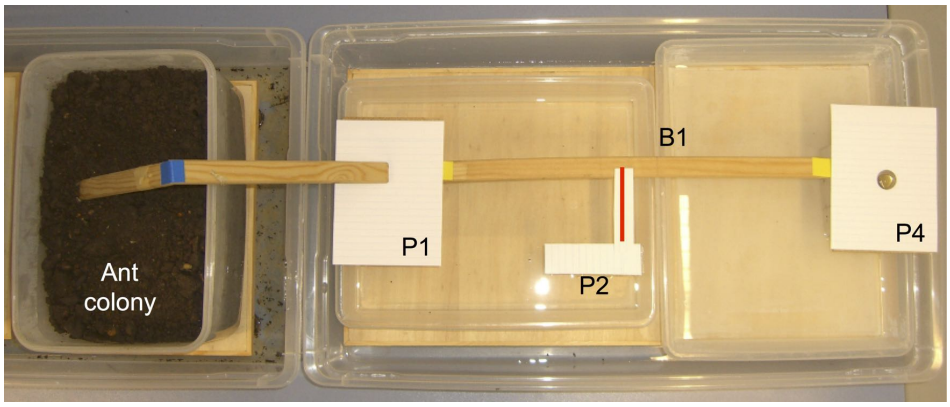


Figure 2.7: Experimental setting of the alternative-trail-branch experiment. An artificial trail (in this figure illustrated by the red line) is applied on a paper bridge branching from natural trail on B1. P1: start platform; P2: inter-platform; P4: food platform; B1: wooden bridge.

and Table A.16 A in Appendix A.1.7.3). To identify differences among gland types within a species the factor *gland type* was nested in the factor *species* (Table A.9 B in Appendix A.1.7.1 and Table A.16 B in Appendix A.1.7.3). To compare species within gland types the factor *species* was nested in the factor *gland type* (Table A.9 C in Appendix A.1.7.1 and Table A.16 C in Appendix A.1.7.3). Statistical significances were tested using random subsets of 9999 permutations of residuals under a reduced model.

2.4.5 Single-point-source experiment

Ants use their glands not only to mark routes to valuable food sources but also to communicate and induce various behaviors in other situations than foraging. In the single-point-source experiment behaviors triggered by highly concentrated gland pheromones were studied. During the trials the bridge connection between start and food platform persisted. Halfway between start and food platform a paper card (10.5 × 15 cm) was attached to the natural trail. A single gland squashed on a little piece of filter paper was placed on the paper card, about 1 cm next to the natural trail. Four gland types and a DCM control were tested in 20 trials each. Treatments alternated between trials. After being connected with the natural trail, the paper card was recorded for two minutes with two digital video cameras (JVC Hybrid, Hard Disk Camcorder Everio): One with an overhead shot of the paper card and the other with a close view of about 3 cm around the filter paper. All behaviors shown by the ants were determined and counted (Table 3.27 in Section 3.3.4).

Data were analyzed using PERMANOVA and PERMANOVA post-hoc pairwise comparisons (PRIMER 6 version 6.1.13 (PRIMER-E Ltd., Ivybridge, UK) extended by the PERMANOVA+ Add-In version 1.0.3). A resemblance matrix was calculated from the data using the Euclidean distances. The PERMANOVA design had two factors, *species* and *gland type*. The factor *gland type* was nested in the factor *species* to analyze differences among gland types within the species (Table A.19 in Appendix A.1.7.4). Statistical significances were tested using a random subset of 9999 permutations of residuals under a reduced model. Mean proportions and standard errors of the three main behaviors shown by a species were ascertained in XLSTAT (version 2016.01.26717, Addinsoft).

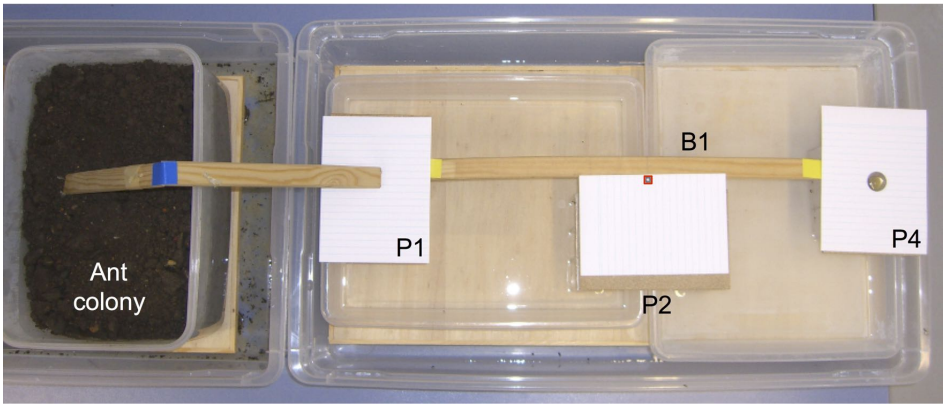


Figure 2.8: Experimental setting of the single-point-source experiment. A single squashed gland (position is illustrated in this figure by the red box) is offered on a paper card next to the natural trail on B1. P1: start platform; P2: inter-platform; P4: food platform; B1: wooden bridge.

2.5 Isolating and identifying gland substances with analytical and preparative gas chromatography

For the isolation of specific trail pheromones, an analytical and preparative gas-chromatograph technique was used: The gland solution is injected into the column of a gas chromatograph (GC) which is coupled with a mass-spectrometer (MS). In the column the analyte is heated so that the volatile substances of the solution evaporate. A carrier gas transports the molecules according to their molecular weight at different velocities through the column. Consequently, the column delivers separated molecules to the MS at different times (i.e. retention time, RT). The MS finally ionizes the molecules and sorts the ions based on their mass-to-charge ratio. Chemicals can then be identified by comparing mass spectra and retention indices (RI) with a target library.

The collection and re-solution of specific fractions of the chromatogram is allowed by a deans switching system, i.e. a branch in the column of the GC which opens by definition. The opening of the deans switch leads the gas flow to a trap where the molecules can be collected with glas vials. To increase concentrations of the analytes, a solid-part microextraction (SPME) technique with a polydimethylsiloxane/ divinylbenzene (PDMS/DVB) fiber was used. The fiber captures the volatile molecules and can directly be inserted into the GC-MS without further dilution by solvents.

2.5.1 Chemical analysis of gland substances

To chemically analyze and compare gland substances, *L. neglectus*, *L. niger* and *L. platythorax* ants were killed via freezing for several minutes. For three gland types, i.e. hindgut, poison gland and Dufour's gland, of each species ten solutions and ten controls were prepared. Glands were dissected in water and solved in DCM containing the internal standard methyl-tridecanoate (MTD) (8.46 µg MTD/ml DCM, FLUKA Analytics, Sigma-Aldrich). Due to problems with detectability of low concentrated pheromones, the solution concentration used for the chemical analyses differed between the gland types: 15 hindguts were solved in 20 µl DCM (≙0.75 GE[8]/µl), ten poison glands were solved in 20 µl DCM (≙0.5 GE/µl), and one Dufour's gland was solved in 100 µl DCM (≙0.01 GE/µl). Forceps were cleaned after transferring the respective gland to the vial. For the control, the cleaned forceps were again dragged through the dissection water and put in the control vial with 20 µl or 100 µl DCM, respectively. 1 µl of the respective gland solution or control was injected to a GC-MS (Agilent 6890N

[8]Definition: One gland equivalent (GE) equals the amount of pheromones in a single gland

gas chromatograph, Agilent 5975 mass spectrometer). Gland ingredients were identified by their mass spectra and retention indices. Peak areas were calculated using the software AMDIS (version 2.68).

For the statistical comparison of *Lasius* glands only substances that were identified in at least 50 percent of the respective gland extracts of *L neglectus*, *L. niger* or *L. platythorax* were considered for analyses. Contaminations in the control were included in any case, even if there was only a single control solution contaminated. The amounts of substances were standardized according to the internal MTD standard. Since different numbers of glands were dissected for the different gland solutions, gland equivalents, i.e. the substance amount in one gland, were calculated for each substance. A resemblance matrix based on Bray-Curtis similarites was calculated from the standardized gland equivalents and analyzed using PERMANOVA (PRIMER 6 version 6.1.13 (PRIMER-E Ltd., Ivybridge, UK) extended by the PERMANOVA+ Add-In version 1.0.3). To analyze differences among species and among gland extracts and controls 18 contrasts for the factor *comparison* were pre-defined in the PERMANOVA design (Table A.21 in Appendix A.1.8). Statistical significances were tested using a random subset of 9999 permutations of residuals under a reduced model.

2.5.2 Isolation and testing of gland substances

To identify species-specific bioactive gland substances full chromatograms of gland solutions were fractioned into defined parts. Each fractional part was then tested for bioactivity. Active fractional parts were fractioned and tested again. This process was repeated until single substances remained.

To find bioactive trail pheromones of the invasive garden ant *L. neglectus*, ants were killed via freezing for several minutes. 20 hindguts were dissected in water and solved in 20 µl DCM. 1 µl of the gland solution was injected to the GC-MS. Fractions of the solution were branched according to retention times determined in prior control GC-MS runs. The full chromatogram was fractioned in one minute intervals from six to 15 minutes retention time (RT). Fractions were collected in vials using the deans switch. To force the condensation of the molecules at the glas walls, all glas vials were previously cooled down with liquid nitrogen. Recaptured samples were solved in 500 µl DCM for the bioassays. Bioactivity of the captured samples was tested in direction-by-choice experiments with *L. neglectus*, *L. niger* and *L. platythorax* (Section 2.4.2). Samples were tested on a significant species-specific attraction to *L. neglectus*. Corresponding retention time intervals of the bioactive fragments were divided in half. The smaller fragments were captured again from the initial gland solution and tested again. The number of experimental trials differed between the fractions (Table 3.33 in Section 3.4.2). Bioactivity data were analyzed using Wilcoxon signed-rank tests in XLSTAT (version 2016.01.26717, Addinsoft).

Due to low substance quantities in hindguts extract concentrations needed to be increased to enable the chemical identification of the bioactive substances: 50 hindguts were dissected and transferred to a vial. After each transfer a PDMS/DVB fiber was put into the vial to capture the volatile substances. For GC-MS analyses the fiber was directly inserted to the GC-MS.

CHAPTER 3

RESULTS

A variety of competitive advantages can explain dominant appearance of pest ant species. In this thesis competitive advantages of the native pest ant *F. fuscocinerea* and of the invasive garden ant *L. neglectus* are evaluated in a field study and in laboratory experiments. The obtained results reveal that the native pest ant *F. fuscocinerea* is able to dominate the two native ant species, *M. ruginodis* and *L. niger*. Several traits known to facilitate competitive dominance in invasive ant species are also present in *F. fuscocinerea*. As for *L. neglectus*, the experiments indicate that this species is able to precisely follow trails, which might support its competitive dominance. Whether this ability is rooted in a more sophisticated pheromone communication remains unclear. However, in the analyses of the gland compounds a species-specific attractant could be identified. This is a first step towards the development of an alternative control strategy for the invasive garden ant *L. neglectus*.

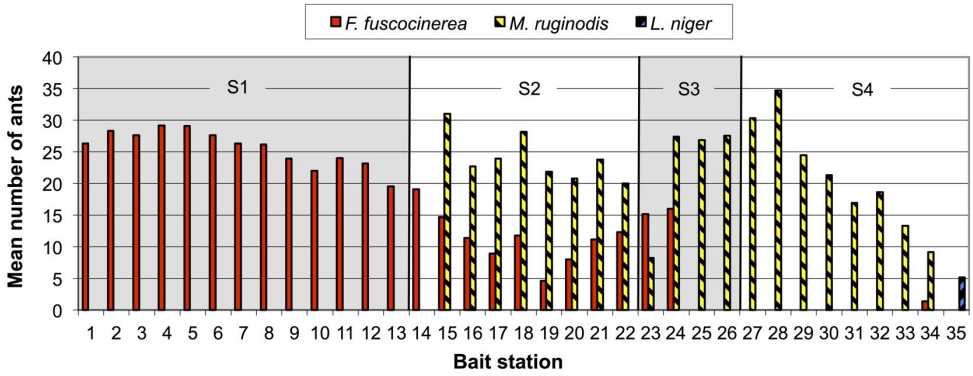
3.1 Results of the field studies

This section is based on Pohl et al. (2018)

Composition and distribution of ant species in an ant community, including the native pest ant species *F. fuscocinerea*, were investigated in a natural environment using bait experiments. Biotic and abiotic habitat characteristics were recorded since they are considered as further factors affecting species distributions (Section 2.2). The field study also comprised repeated ant counts of *F. fuscocinerea* on conifer trunks to estimate foraging activity in the course of the day (Section 2.2.2).

According to differences in vegetation and substrate, the study area was divided into four sections (S1 - S4) (Figure 3.1): S1 was characterized by a sandy substrate, multiple high conifers (Norway spruce *Picea abies*) and several scattered lower deciduous trees (European white birch *Betula pendula*, white willow *Salix alba* and field maple *Acer campestre*). Tree cover in S1 was about 50 %, whereas plant cover only was about 10 % and included mainly reeds, grasses and herbs. The sun exposure of the bait stations varied between 10 % and 100 %. S2 was only scarcely covered with trees and the white willow *S. alba* dominated the vegetation. The scarce plant cover consisted of reeds and herbs and was found on a sand/gravel substrate next to the gravel path. The sun exposure of the bait stations in S2 was with 90 % - 100 % very high. In S3 the substrate changed to soil. Higher field maples were found next to white willows. Tree cover was less than in S2 (S2: 30 %; S3: 20 %). In contrast, in S3 the plant cover which consisted of grasses and reeds was the highest in the habitat (~ 75 %). Sun exposure of the bait stations was, thus, reduced to about 15 %. S4 was

characterized by a dense vegetation consisting of high deciduous trees and bushes (Norway maple *Acer platanoides*, field maple *A. campestre*, white birch *B. pendula*, common hornbeam *Carpinus betulus*, common dogwood *Cornus sanguinea*, common hazel *Corylus avellana*, common hawthorn *Crataegus monogyna*, European beech *Fagus sylvatica*, European ash *Fraxinus excelsior*, European oak *Quercus robur*, common buckthorn *Rhamnus cathartica*, white willow *S. alba* and tall goldenrod *Solidago gigantea*). Stones and leaves were found in and on the soil. As in S3 sun exposure of the bait stations was reduced in S4.



Section	S 1		S 2	S 3		S 4
Length	~ 145 m		~ 105 m	~ 45 m		~ 105 m
Bait station	1 - 13		14 - 22	23 - 26		27 - 35
Substrate	sand		sand, gravel	soil		soil, stones, leaves
Tree cover	40 %	10 %	30 %	20 %		60 %
Trees	conifers	deciduous trees	deciduous trees	deciduous trees		deciduous trees
Tree height	~ 15 m	~ 7 m	~ 3 m	~ 12 m		~ 12 m
Plant cover	10 %		20 %	60 %	15 %	10 %
Plants	reed, herbs, grasses		reed, herbs	grass	reed	reed, meadow, bushes
Sun exposure	10 - 100 %		90 - 100 %	15 %		15 %

Figure 3.1: Bar chart: Distribution of *F. fuscocinerea* (red bars), *M. ruginodis* (yellow/black bars) and *L. niger* (blue/black bars) in the study area. The figure shows the mean numbers of ants counted at 35 bait stations. Please note, that *L. niger* ants occurred only at bait station no. 35. Table: Characterization of the study area: The habitat was divided into four sections (S1 - S4) according to differences in substrate and vegetation. Adapted from Pohl et al. (2018).

3.1.1 Species occurrence and abundance in a natural habitat

Three ant species, *F. fuscocinerea*, *M. ruginodis* and *L. niger*, visited the baits during the field study. Species composition at the baits differed throughout the habitat (Figure 3.1): In S1 only *F. fuscocinerea* visited the baits. In S2 and S3 the occurrences of *F. fuscocinerea* and of *M. ruginodis* overlapped, yet only spatially, not temporally. In S4 *F. fuscocinerea* appeared only at one bait station while *M. ruginodis* dominated the baits in this section. *Lasius niger* occurred only at one bait station at the edge of S4.

Numbers of ants at the baits were significantly influenced by the recorded biotic and abiotic habitat parameters (see effects of factors and covariates in Table 3.1). The influence of temperature varied among the species (Figure 3.2): There was no clear increase or decrease in the mean number of *F. fuscocinerea* workers (Pearson correlation: $N = 49$, $R = -0.218$, $P = 0.133$) and in the mean number of *M. ruginodis* workers at the baits (Pearson correlation: $N = 50$, $R = -0.145$, $P = 0.317$). In contrast, although the evidence is weak, mean worker numbers of *L. niger* increased with higher temperatures (Pearson correlation: $N = 18$, $R = 0.472$, $P = 0.048$).

It seems that the ants could freely choose between honey and tuna at the bait stations, as there was always only one species at a bait station at any given time, except in a single observation. Species-specific preferences in bait choice is obvious: *Formica fuscocinerea* and *L. niger* preferred tuna baits, *M. ruginodis* preferred honey baits (Table 3.2; Figure 3.3).

Table 3.1: Statistical analysis of the field studies investigating species composition of *F. fuscocinerea*, *L. niger* and *M. ruginodis* in a natural habitat: PERMANOVA analysis of the influence of biotic and abiotic parameters on the ant numbers of the three different species counted at honey and tuna baits at thirty-five bait stations in four different sections of a habitat. The PERMANOVA design took six factors (*date*, *time*, *bait station*, *sun exposure* and *bait type*) and two covariates (*humidity* and *temperature*) into account. The table shows the degrees of freedom (df), sums of squares (SS), mean squares (MS), Pseudo-F values, P-values determined by permutations, and the numbers of unique permutations. P-values less than 0.05 are marked in bold.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P (perm)**	**Unique perms**
Humidity[1]	1	14990	14990	2.4515	**0.045**	9931
Temperature[2]	1	$3.76 \cdot 10^5$	$3.76 \cdot 10^5$	4.1956	**0.002**	9932
Section[3]	3	$2.15 \cdot 10^6$	$7.18 \cdot 10^5$	24.144	**< 0.001**	9936
Date of observation[4]	4	40355	10089	7.1376	**< 0.001**	9916
Time of observation[5]	6	18989	3164.9	2.1493	**< 0.001**	9893
Bait station (section)[6]	31	$9.54 \cdot 10^5$	30763	25.497	**< 0.001**	9766
Sun exposure (section)[7]	8	31927	3990.9	3.3078	**< 0.001**	9862
Bait type (bait station (section))[8]	35	$7.65 \cdot 10^5$	21847	18.108	**< 0.001**	9761
Residuals	1961	$2.37 \cdot 10^6$	1206.5			
Total	2050	$6.72 \cdot 10^6$				

[1] Relative air humidity was measured at one shady point in the habitat every time of observation; [2] temperature was measured at every bait station every time of observation; [3] the habitat was divided into four sections according to differences in substrate and vegetation; [4] observations were made on five days (28^{th}/30^{th} June, 2^{nd}/6^{th}/9^{th} July); [5] observations were made seven times a day (at 8:30 a.m., 9:30 a.m., 10:30 a.m., 3:30 p.m., 4:30 p.m., 8:30 p.m., 9:30 p.m.); [6] thirty-five bait stations were observed; every bait station was assigned to one of the four habitat sections, hence *bait station* was nested in *section*; [7] sun exposure was estimated at every bait station every time of observation and assigned to one of the three categories sunny, semi-shady and shady; sun exposure was assigned to one of the four habitat sections, hence *sun exposure* was nested in *section*; [8] every bait station contained two bait types: honey and tuna; every bait was assigned to a bait station, hence *bait type* was nested in *bait station*.

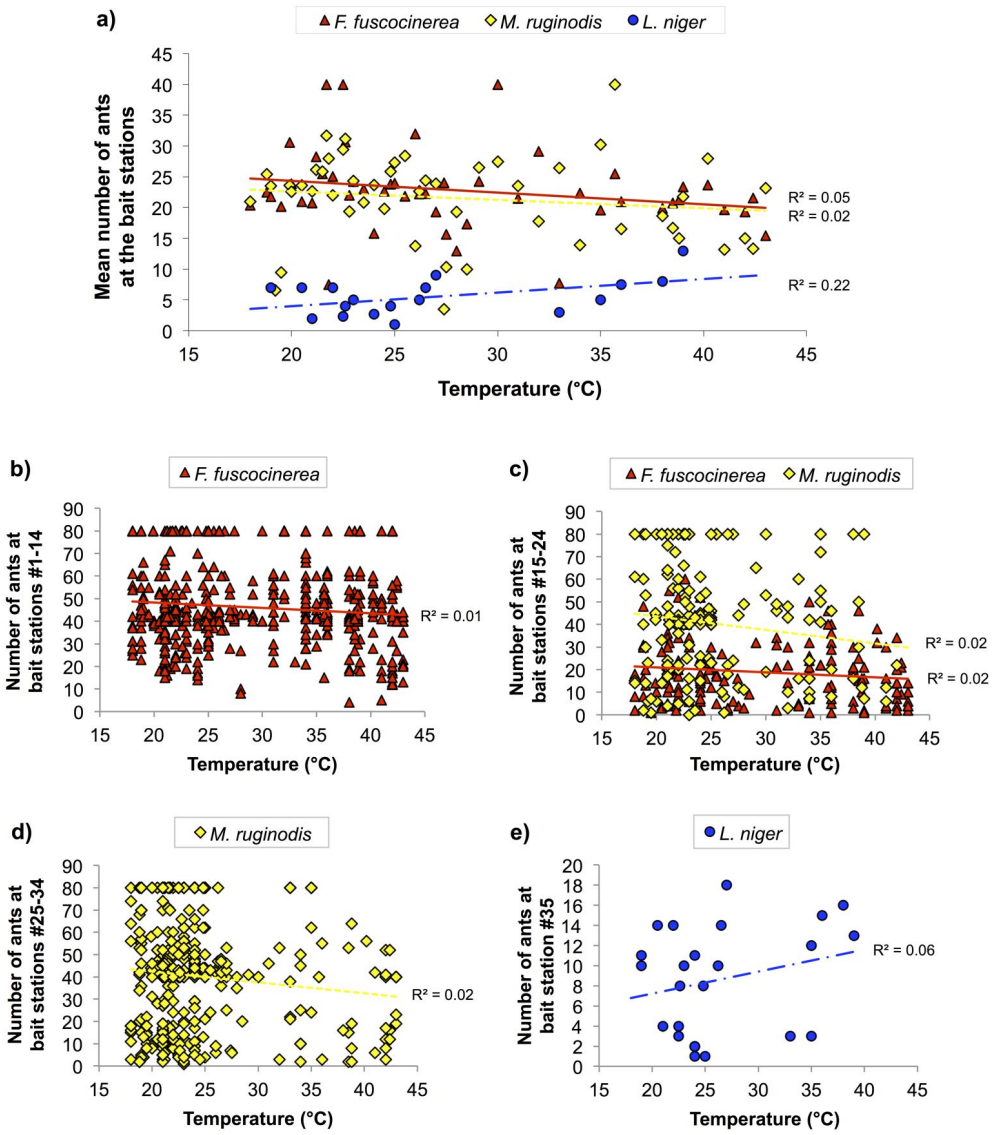


Figure 3.2: Effect of temperature on ant numbers reported at the baits in the field study. The figure shows a) the mean numbers of *F. fuscocinerea*, *M. ruginodis* and *L. niger* ants averaged over all counts at the respective temperature and b)-e) all counted numbers of ants at the respective temperatures separated by bait stations according to the occurrence of the species.

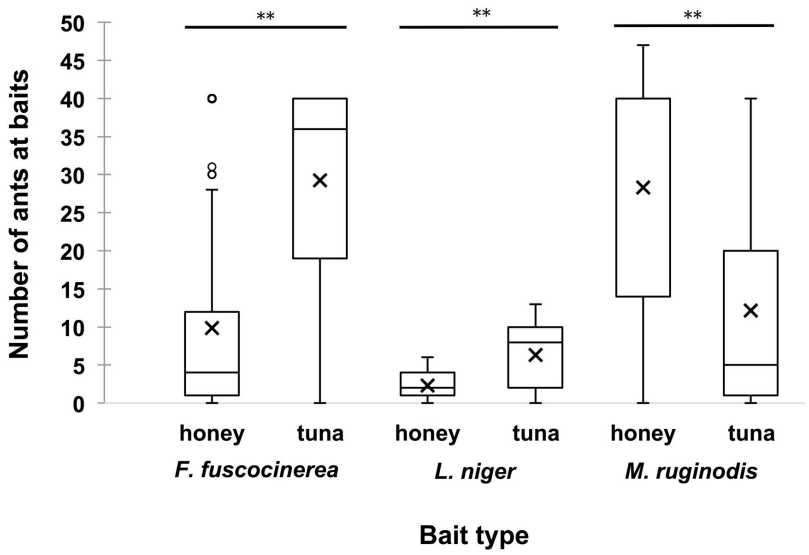


Figure 3.3: Bait choice of three ant species during the field study: Number of *F. fuscocinerea*, *M. ruginodis* and *L. niger* ants that were counted at honey or tuna baits. The figure shows box-and-whisker plots with first and third quartiles, median and whiskers (minimum and maximum value); mean (×); values that are more than 1.5 times the interquartile range (IQR) are displayed as outliers (∘). Significant differences between groups: Wilcoxon signed-rank test $P \leq 0.001$ (∗∗).

Table 3.2: Post-hoc Wilcoxon signed-rank test, testing the effect of *bait type* within *F. fuscocinerea*, *L. niger* and *M. ruginodis*: Comparison of the numbers of ants counted at honey baits with the numbers of ants counted at tuna baits. The table shows the numbers of observations (N_{honey}, N_{tuna}), the test statistics (W) and two-sided P-values. P-values less than 0.05 are marked in bold.

Species	$\mathbf{N_{honey}}$	$\mathbf{N_{tuna}}$	W	**two-sided** P
F. fuscocinerea	655	655	2678.5	**< 0.001**
M. ruginodis	488	488	80211.0	**< 0.001**
L. niger	24	24	9.5	**< 0.001**

3.1.2 Foraging activity of *F. fuscocinerea*

The foraging activity of *F. fuscocinerea* was observed on seven different spruce trunks in habitat section S1, which was the section with the highest *F. fuscocinerea* density. Ants were counted considering whether they move up or down the trunks several times throughout the day and night. Distended abdomens, visible by the transparent intersegmental membranes, characterized ants returning to the nests from the tree canopies.

On tree trunks *F. fuscocinerea* was equally active during day and night (see effect of *daytime* in Table 3.3). The number of ants going up to forage and the number of ants returning from the tree canopies did neither differ overall nor between day and night times (see effects of *direction* and *daytime* × *direction* in Table 3.3). The only difference in ant activity was found among trees and among trees at different daytimes (see effects of *tree* and *daytime* × *tree* in Table 3.3).

Table 3.3: *Formica fuscocinerea* foraging activity over the course of a day: PERMANOVA analysis of the ant numbers going up or down on seven different spruce trunks. The PERMANOVA design took three factors (daytime, tree and direction) and their interactions into account. The table shows the degrees of freedom (df), sums of squares (SS), mean squares (MS), Pseudo-F values, P-values determined by permutations, and the numbers of unique permutations. P-values less than 0.05 are marked in bold.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P (perm)**	**Unique perms**
Daytime[1]	1	1991.8	1991.8	2.2916	0.151	9938
Tree[2]	6	72691	12115	85.423	**< 0.001**	9948
Direction[3]	1	314.78	314.78	2.1263	0.158	9935
Daytime × tree[4]	6	5215.1	869.18	6.1285	**< 0.001**	9932
Daytime × direction[5]	1	159.63	159.63	1.1255	0.300	9954
Tree × direction[6]	6	888.23	148.04	1.0438	0.415	9941
Residuals	34	4822.1	141.83			
Total	55	86083				

[1]Counts repeated at four different times were assigned to two daytimes: day (10:00 a.m., 3:00 p.m.), night (11:00 p.m., 12:00 p.m.); [2]foraging activity was observed on seven different spruce trunks; [3]ants were distinguished whether they go up or down the tree trunk. Analysis of interactions among factors: [4]differences in ant numbers on different trees at different daytimes; [5]differences in ant numbers going up or down at different daytimes; [6]differences in ant numbers going up or down at different trees.

3.2 Inter- and intraspecific competition experiments

Some ant species are highly aggressive and some ant species are less aggressive when they encounter non-colony members at their food sources. Interference competitors engage in direct confrontation. Exploitative competitors rather avoid direct encounters and score points with speed, i.e. through a faster exploitation of resources. The competitive strategy of the ant species was investigated with an exploitative and interference competition (EIC) experiment (Section 2.3.1). The EIC experiment consists of two parts: In the first part one species (i.e. explorer species) has exclusive access to the food source. At the onset of the second part a second species (i.e. competitor species) is given concurrent access. Ant numbers at the food source are regularly recorded during both parts of the experiment.

Aggression is a further measurement for the interference competition ability of an ant species. Aggression indices were determined in one-on-one aggression tests and in aggression tests with group of ants (Section 2.3.2). While in one-on-one aggression tests with only two ants coming together the critical factor is the aggression ability of each individual, in aggression tests with group of ants numerical superiority or inferiority can influence the species' competitive ability in encounters. Since ants are often also aggressive against conspecific non-colony members aggression tests can be used to identify colony membership of ant individuals.

3.2.1 Exploitative and interference competition experiments with *F. fuscocinerea*, *M. ruginodis* and *L. niger*

This section is based on Pohl et al. (2018)

In the first part of the EIC experiment, i.e. in the absence of a competitor, equivalent total numbers of *F. fuscocinerea*, *M. ruginodis* and *L. niger* ants occurred at the food source (see effects of *C1*, *C2* and *C3* in Table 3.4; Figure 3.4). While *F. fuscocinerea* and *M. ruginodis* also showed similar temporal changes of ant numbers (see effect of *time* × *C1* in Table 3.4; Figure 3.5 c, d Part 1), the temporal change of *L. niger* ant numbers differed from those of the other two species (see effects of *time* × *C2* and *time* × *C3* in Table 3.4; Figure 3.5 b, c, d Part 1).

In the second part of the EIC experiment, i.e. in presence of a competitor, only small numbers of *L. niger* and *M. ruginodis* ants showed up at food sources that were already occupied by *F. fuscocinerea*. Total ant numbers of *L. niger* and *M. ruginodis* were similar in this case (see effect of *C4* in Table 3.5; Figure 3.4). Interestingly, the presence of *M. ruginodis* at the food source had a similar effect on *F. fuscocinerea*: Total ant numbers of *F. fuscocinerea* and *M. ruginodis* did not differ, when they showed up at a food source occupied by the respective other ant species (see effect of *C2* in Table 3.5; Figure 3.4). In contrast to food sources that were occupied by *M. ruginodis*, more *F. fuscocinerea* ants showed up at food sources that were occupied by *L. niger* (see effect of *C1* in Table 3.5; Figure 3.4). Hence, total numbers of *F. fuscocinerea* ants depended on the explorer species that was already present at the food source.

The three ant species differed in the temporal changes of ant numbers when the food source was already occupied by an explorer species (see effects of *time* × *C2*, *time* × *C3* and *time* × *C4* in Table 3.5; Figure 3.5 Part 2). In *F. fuscocinerea* the temporal change of ant numbers depended on the explorer species, with more ants appearing per minute at food sources already occupied by *L. niger* compared to food sources already occupied by *M. ruginodis* (see effect of *time* × *C1* in Table 3.5; Figure 3.5 b, d Part 2). For all species combinations the numbers of explorer and competitor ants at the food sources were negatively correlated (Table 3.7; Figure 3.5 Part 2). That means, that an increasing number of competitor ants was accompanied with a decreasing number of explorer ants.

In all cases except one (see below) a higher total number of ants showed up at unoccupied food sources (part 1) compared to food sources that were already occupied by an explorer species (part 2) (see effects of *C1*, *C3* and *C4* in Table 3.6; Figure 3.4). This was also evident in the temporal changes

of ant numbers (see effects of *time* × *C1*, *time* × *C2*, *time* × *C3* and *time* × *C4* Table 3.6; Figure 3.5). An exception was *F. fuscocinerea* whose total ant numbers did not differ between unoccupied food sources and food sources that were occupied by *L. niger* (see effect of *C2* in Table 3.6; Figure 3.5 a, b). Actually, more ants showed up per time in the presence of *L. niger* (see effect of *time* × *C2* in Table 3.6; Figure 3.5 a, b).

Table 3.4: Exploitative and interference competition (EIC) experiment comprising two parts carried out with species pairs of the species *F. fuscocinerea, M. ruginodis* and *L. niger*. Part 1: Only the explorer species was given access to the food source. PERMANOVA analyses with ant numbers of the explorer species as response variable. The PERMANOVA design took three factors (*time, explorer species* and *trial number*) and the interaction between *time* and *explorer species* into account. The table shows the degrees of freedom (df), sums of squares (SS), mean squares (MS), Pseudo-*F* values, *P*-values determined by permutations, and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

Source	**df**	**SS**	**MS**	**Pseudo-*F***	***P* (perm)**	**Unique perms**
Time[1]	1	23821	23821	716.97	**< 0.001**	9847
Explorer species[2]	2	842.9	421.45	0.23678	0.790	9951
C1[3]: *F. fuscocinerea* vs. *M. ruginodis*	1	132.25	132.25	$6.39 \cdot 10^2$	0.804	5743
C2: *F. fuscocinerea* vs. *L. niger*	1	839.07	839.07	0.50866	0.485	5490
C3: *M. ruginodis* vs. *L. niger*	1	228.81	228.81	0.14917	0.693	3101
Trial number (expl. spec.)[4]	37	65857	1779.9	53.572	**< 0.001**	9876
Trial number (C1)[5]	28	57917	2068.5	60.591	**< 0.001**	9903
Trial number (C2)	28	46188	1649.6	54.429	**< 0.001**	9882
Trial number (C3)	18	27610	1533.9	42.326	**< 0.001**	9911
Time × explorer species[6]	2	433.47	216.74	65.233	**0.002**	9942
Time × C1[7]	1	0.5372	0.5372	$1.57 \cdot 10^2$	0.901	9845
Time × C2	1	375.3	375.3	12.383	**< 0.001**	9823
Time × C3	1	303.18	303.18	8.366	**0.003**	9849
Residuals	557	18506	33.225			
Total	599	$1.09 \cdot 10^9$				

[1]Ant numbers at the food source were counted every minute for 15 minutes; [2]*F. fuscocinerea*,*M. ruginodis* and *L. niger*; [3]comparisons of interest were pre-defined as contrasts (C1 - C3); [4]analysis of differences between trials within each explorer species, [5]and for pairwise comparisons of explorer species; [6]analysis of interactions among factors: analysis of the differences in the temporal change of ant numbers over all explorer species [7]and for pairwise comparisons of explorer species.

Table 3.5: Exploitative and interference competition (EIC) experiment comprising two parts carried out with species pairs of the species *F. fuscocinerea* (FF), *M. ruginodis* (MR) and *L. niger* (LN). Part 2: the explorer species and an additional competitor species were given access to the food source. PERMANOVA analyses with ant numbers of the competitor species as response variable. The respective explorer species is given as subscript. The PERMANOVA design took three factors (*time*, *competitor species* and *trial number*) and the interaction between *time* and *competitor species* into account. The table shows the degrees of freedom (df), sums of squares (SS), mean squares (MS), Pseudo-*F* values, *P*-values determined by permutations, and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

Source	**df**	**SS**	**MS**	**Pseudo-*F***	***P* (perm)**	**Unique perms**
Time[1]	1	7142	7142	291.64	**< 0.001**	9834
Competitor species[2]	3	36093	12031	13.549	**< 0.001**	9949
C1[3]: FF_{MR} vs. FF_{LN}	1	18728	18728	11.502	**0.004**	9201
C2: MR_{FF} vs. FF_{MR}	1	330.8	330.8	0.68506	0.335	7729
C3: FF_{LN} vs. LN_{FF}	1	27957	27957	21.616	**< 0.001**	2954
C4: LN_{FF} vs. MR_{FF}	1	144.45	144.45	0.98232	0.427	762
Trial number (comp. spec.)[4]	36	31966	887.95	36.373	**< 0.001**	9876
Trial number (C1)[5]	18	29308	1628.2	37.551	**< 0.001**	9913
Trial number (C2)	18	8691.9	482.88	26.69	**< 0.001**	9916
Trial number (C3)	18	23280	1293.3	42.084	**< 0.001**	9904
Trial number (C4)	18	2647	147.05	26.909	**< 0.001**	9913
Time × competitor species[6]	3	7983.8	2661.3	109.01	**< 0.001**	9951
Time × C1[7]	1	3160	3160	72.88	**< 0.001**	9827
Time × C2	1	207.48	207.48	11.468	**< 0.001**	9821
Time × C3	1	6767	6767	220.19	**< 0.001**	9831
Time × C4	1	131.19	131.19	24.006	**< 0.001**	9848
Residuals	596	14550	24.412			
Total	639	97735				

[1]Ant numbers at the food source were counted every minute for 15 minutes; [2]*F. fuscocinerea* (FF), *M. ruginodis* (MR) and *L. niger* (LN). [3]comparisons of interest were pre-defined as contrasts (C1 - C4): *F. fuscocinerea* vs. *F. fuscocinerea, M. ruginodis* vs. *F. fuscocinerea, F. fuscocinerea* vs. *L. niger* and *L. niger* vs. *M. ruginodis*; the respective explorer species is given as subscript; [4]analysis of differences between trials within each competitor species, [5]and for pairwise comparisons of competitor species; [6]analysis of interactions among factors: analysis of the differences in the temporal change of ant numbers over all competitor species [7]and for pairwise comparisons of competitor species;

Table 3.6: Exploitative and interference competition (EIC) experiment comprising two parts carried out with species pairs of the species *F. fuscocinerea* (FF), *M. ruginodis* (MR) and *L. niger* (LN). Part 1: Only the explorer species was given access to the food source. Part 2: The explorer species and an additional competitor species were given access to the food source. Comparison of the number of ants when the species was the explorer species (part 1) with the number of ants when the species was the competitor species (part 2): PERMANOVA analyses with explorer ant numbers (part 1) and competitor ant numbers (part 2) of each species as response variable. The respective explorer species during part 2 is given as subscript. The PERMANOVA design took three factors (*time*, *group* and *trial number*) and the interaction between *time* and the pre-defined comparisons for each species (contrasts) into account. The table shows the degrees of freedom (df), sums of squares (SS), mean squares (MS), Pseudo-F values, P-values determined by permutations, and the numbers of unique permutations. P-values less than 0.05 are marked in bold.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P (perm)**	**Unique perms**
Time[1]	1	27528	27528	964.65	**< 0.001**	9821
Group[2]	6	54711	9118.5	7.0442	**< 0.001**	9954
C1[3]: FF vs. FF_{MR}	1	14795	14795	9.5816	**0.005**	5363
C2: FF vs. FF_{LN}	1	763.6	763.6	0.35843	0.548	5708
C3: MR vs. MR_{FF}	1	12378	12378	10.225	**0.006**	2514
C4: LN vs. LN_{FF}	1	11334	11334	25.286	**< 0.001**	1912
Trial number (group)[4]	73	94496	1294.5	45.361	**< 0.001**	9824
Trial number (C1)[5]	28	43234	1544.1	54.058	**< 0.001**	9900
Trial number (C2)	28	59651	2130.4	53.377	**< 0.001**	9906
Trial number (C3)	18	21790	1210.6	47.269	**< 0.001**	9918
Trial number (C4)	18	8068.6	448.25	27.995	**< 0.001**	9911
Time × group[6]	6	10587	1764.5	61.832	**< 0.001**	9947
Time × C1[7]	1	995.23	995.23	34.844	**< 0.001**	9815
Time × C2	1	1098	1098	27.512	**< 0.001**	9812
Time × C3	1	1539.3	1539.3	60.106	**< 0.001**	9832
Time × C4	1	4469.6	4469.6	279.14	**< 0.001**	9854
Residuals	1113	31761	28.537			
Total	1199	$2.19 \cdot 10^5$				

[1]Ant numbers at the food source were counted every minute for 30 minutes; [2]*F. fuscocinerea* (FF), *M. ruginodis* (MR), *L. niger* (LN), *F. fuscocinerea*$_{M.\ ruginodis}$ (FF_{MR}), *F. fuscocinerea*$_{L.\ niger}$ (FF_{LN}), *M. ruginodis*$_{F.\ fuscocinerea}$ (MR_{FF}) and *L. niger*$_{F.\ fuscocinerea}$ (LN_{FF}); the respective explorer species is given as subscript; [3]comparisons of interest were pre-defined as contrasts (C1 - C4); [4]analysis of differences between trials within the groups, [5]and for pairwise comparisons of the two parts of the experiment for each species; [6]analysis of interactions among factors: analysis of the differences in the temporal change of ant numbers over all groups [7]and for pairwise comparisons of of the two parts of the experiment for each species.

Table 3.7: Exploitative and interference competition (EIC) experiment comprising two parts carried out with species pairs of *F. fuscocinerea, M. ruginodis* and *L. niger*. Part 2: the explorer species and an additional competitor species were given access to the food source. Spearman rank correlations between the number of ants of the explorer species and the number of ants of the competitor species. The table shows the numbers of observations (N), Spearman's ρ-values, and P-values. P-values less than 0.05 are marked in bold.

Explorer species	**Competitor species**	**N**	ρ	P
F. fuscocinerea	*L. niger*	160	-0.457	**< 0.001**
L. niger	*F. fuscocinerea*	160	-0.819	**< 0.001**
F. fuscocinerea	*M. ruginodis*	160	-0.685	**< 0.001**
M. ruginodis	*F. fuscocinerea*	160	-0.814	**< 0.001**

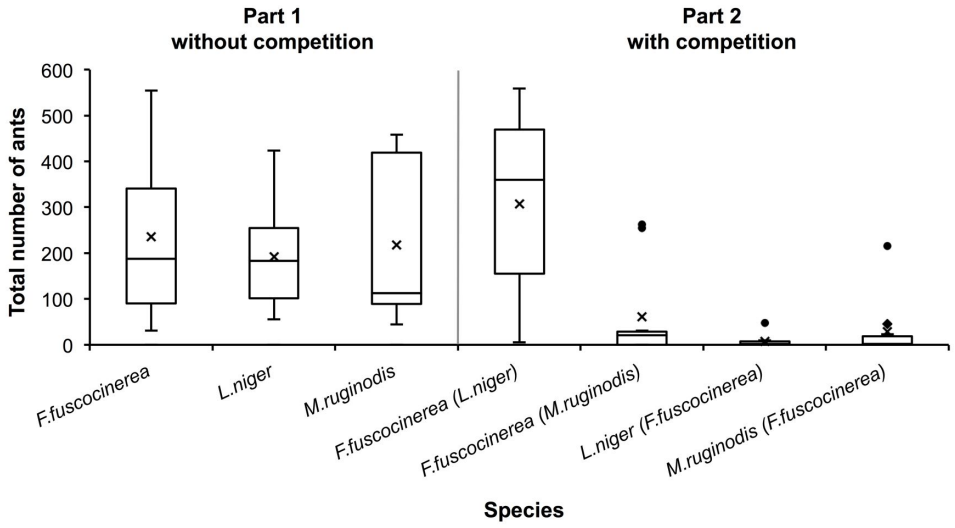


Figure 3.4: Total numbers of ants that were present at the food source during the exploitative and interference competition experiment. Part 1: explorer species in the absence of competition during the first part of the experiment. Part 2: Competitor species during the second part of the experiment. The respective explorer species that had occupied the food source is given in brackets. The figure shows box-and-whisker plots with first and third quartiles, median and whiskers (minimum and maximum value); mean (×); values that are more than 1.5 times the interquartile range (IQR) are displayed as outliers (◆), values that are more than 3 IQR are displayed as extreme points(●). Adapted from Pohl et al. (2018).

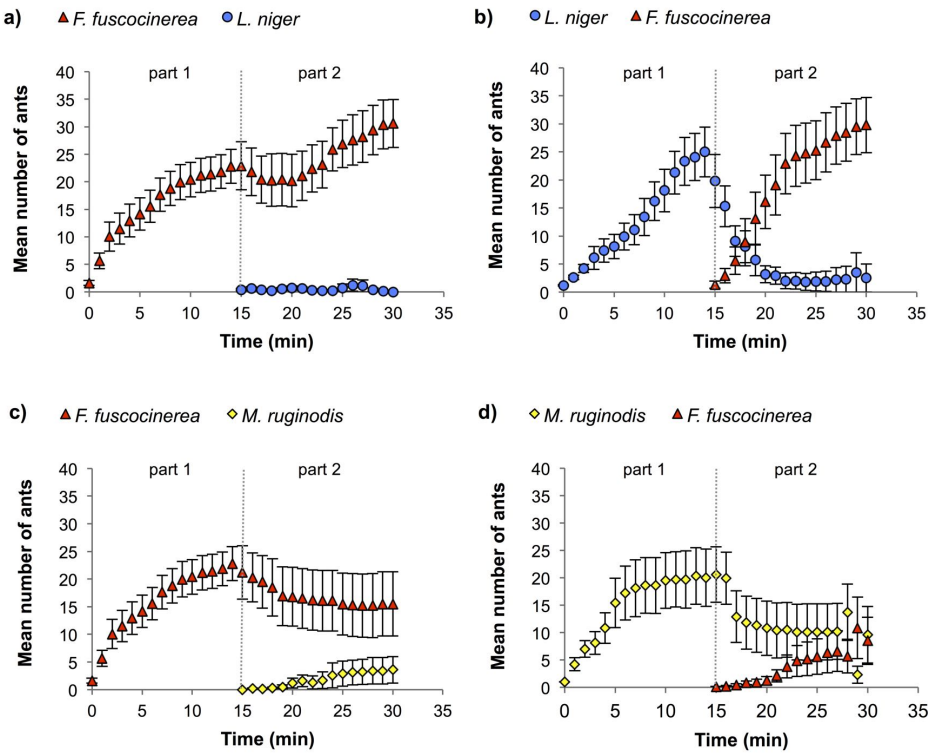


Figure 3.5: Exploitative and interference competition experiment. Mean numbers and standard errors of ants at the food source are displayed. Part 1: explorer species in the absence of competition during the first part of the experiment (minutes 0 – 14). Part 2: Competitor species during the second part of the experiment (minutes 15 – 30). Source: Pohl et al. (2018)

3.2.2 Exploitative and interference competition experiments with *L. neglectus*, *L. niger* and *L. platythorax*

The invasive garden ant *L. neglectus* needed more time to discover the food sources compared to the native species, *L. niger* and *L. platythorax* (Table 3.8). The discovery times of the native species were similar (Table 3.8).

In the first part of the EIC experiment, i.e. in the absence of a competitor, equivalent total numbers of *L. neglectus*, *L. niger* and *L. platythorax* ants occurred at the food source (see effects of *C1*, *C2* and *C3* in Table 3.9; Figure 3.6 Part 1). The species, however, differed in the temporal changes of ant numbers (see effects of *time* × *C1*, *time* × *C2* and *time* × *C3* in Table 3.9; Figure 3.7 b, c, d Part 1). In the second part of the EIC experiment, only small numbers of *L. neglectus*, *L. niger* and *L. platythorax* ants showed up at the food when it was already occupied by an explorer species. Total ant numbers of the three ant species were similar, except in one case: More *L. platythorax* than *L. niger* individuals occurred at the food source when the invasive garden ant *L. neglectus* already was on site as the explorer species (see effects of *C1*, *C2*, *C3* and *C4* in Table 3.10; Figure 3.6 Part 2). Furthermore, the three species differed in the temporal changes of ant numbers at the food source (see effects of *time* × *C1*, *time* × *C2*, *time* × *C3* and *time* × *C4* in Table 3.10; Figure 3.7 Part 2). For all species combinations the numbers of explorer and competitor ants were negatively correlated (Table 3.12; Figure 3.7 Part 2).

In all cases more ants reached unoccupied food sources (part 1) than food sources that were already occupied by an explorer species (part 2) (see effects of *C1*, *C2*, *C3* and *C4* in Table 3.11;

Figure 3.6). This was also evident in the temporal changes of ant numbers (see effects of *time* × *C1*, *time* × *C2*, *time* × *C3* and *time* × *C4* in Table 3.11; Figure 3.7).

Table 3.8: Comparison of the food discovery time of *L. neglectus*, *L. niger* and *L. platythorax*. Post-hoc pairwise comparison with Dunn's procedure. The table shows pairwise differences ($W_{i,j}$) and two-sided *P*-values. The significance level is Bonferroni corrected: *P*-values less than 0.017 are marked in bold.

Comparison			$\mathbf{W_{i,j}}$	*P*
L. neglectus	vs.	*L. niger*	26.413	**< 0.001**
L. neglectus	vs.	*L. platythorax*	19.438	**0.002**
L. niger	vs.	*L. platythorax*	-6.975	0.342

Table 3.9: Exploitative and interference competition (EIC) experiment comprising two parts carried out with species pairs of the invasive species *L. neglectus*, and the two native species, *L. niger* and *L. platythorax*. Part 1: Only the explorer species was given access to the food source. PERMANOVA analyses with ant numbers of the explorer species as response variable. The PERMANOVA design took three factors (*time*, *explorer species* and *trial number*) and the interaction between *time* and *explorer species* into account. The table shows the degrees of freedom (df), sums of squares (SS), mean squares (MS), Pseudo-*F* values, *P*-values determined by permutations, and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

Source	**df**	**SS**	**MS**	**Pseudo-*F***	***P* (perm)**	**Unique perms**
Time[1]	1	$2.53 \cdot 10^9$	$2.53 \cdot 10^9$	6783.6	**< 0.001**	9833
Explorer species[2]	2	4179.6	2089.8	0.81856	0.448	9957
C1[3]: *L. neglectus* vs. *L. niger*	1	1026.1	1026.1	0.57053	0.464	7837
C2: *L. neglectus* vs. *L. platythorax*	1	1786.5	1786.5	0.68797	0.411	8019
C3: *L. niger* vs. *L. platythorax*	1	4140.4	4140.4	11.381	0.296	6664
Trial number (expl. species)[4]	77	$1.97 \cdot 10^9$	2553	68.349	**< 0.001**	9829
Trial number (C1)[5]	58	$1.04 \cdot 10^9$	1798.6	59.283	**< 0.001**	9853
Trial number (C2)	58	$1.51 \cdot 10^9$	2596.7	64.686	**< 0.001**	9840
Trial number (C3)	38	$1.38 \cdot 10^9$	3637.9	83.261	**< 0.001**	9887
Time × explorer species[6]	2	3744.9	1872.5	50.129	**< 0.001**	9950
Time × C1[7]	1	3285.8	3285.8	108.31	**< 0.001**	9843
Time × C2	1	1545.2	1545.2	38.491	**< 0.001**	9804
Time × C3	1	243.37	243.37	5.57	**0.018**	9830
Residuals	2317	86548	37.353			
Total	2399	$5.44 \cdot 10^9$				

[1]Ant numbers at the food source were counted every 30 seconds for 14.5 minutes; [2]*L. neglectus*, *L. niger* and *L. platythorax*; [3]comparisons of interest were pre-defined as contrasts (C1 - C3); [4]analysis of differences between trials within each explorer species, [5]and for pairwise comparisons of explorer species; [6]analysis of interactions among factors: analysis of the differences in the temporal change of ant numbers over all explorer species [7]and for pairwise comparisons of explorer species.

Table 3.10: Exploitative and interference competition (EIC) experiment comprising two parts carried out with species pairs of the invasive species *L. neglectus* (LNE), and the two native species, *L. niger* (LN) and *L. platythorax* (LP). Part 2: the explorer species and an additional competitor species were given access to the food source. PERMANOVA analyses with ant numbers of the competitor species as response variable. The respective explorer species is given as subscript. The PERMANOVA design took three factors (*time, competitor species* and *trial number*) and the interaction between *time* and *competitor species* into account. The table shows the degrees of freedom (df), sums of squares (SS), mean squares (MS), Pseudo-F values, P-values determined by permutations, and the numbers of unique permutations. P-values less than 0.05 are marked in bold.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P (perm)**	**Unique perms**
Time[1]	1	11304	11304	876.9	**< 0.001**	9833
Competitor species[2]	3	6746.2	2248.7	3.2376	**0.026**	9960
C1[3]: LN_{LNE} vs. LNE_{LN}	1	721.71	721.71	2.3827	0.139	3716
C2: LP_{LNE} vs. LNE_{LP}	1	180.43	180.43	0.1661	0.685	5265
C3: LN_{LNE} vs. LP_{LNE}	1	5506.3	5506.3	7.9666	**0.007**	4689
C4: LNE_{LN} vs. LNE_{LP}	1	1149.7	1149.7	1.6473	0.211	4714
Trial number (comp. spec.)[4]	76	52787	694.56	53.879	**< 0.001**	9849
Trial number (C1)[5]	38	11510	302.89	51	**< 0.001**	9882
Trial number (C2)	38	41277	1086.2	54.741	**< 0.001**	9899
Trial number (C3)	38	26265	691.17	42.005	**< 0.001**	9873
Trial number (C4)	38	26522	697.95	74.825	**< 0.001**	9879
Time × competitor species[6]	3	2514.6	838.2	65.021	**< 0.001**	9957
Time × C1[7]	1	314.21	314.21	52.906	**< 0.001**	9839
Time × C2	1	175.38	175.38	8.838	**0.003**	9849
Time × C3	1	2237.7	2237.7	135.99	**< 0.001**	9819
Time × C4	1	266.85	266.85	28.608	**< 0.001**	9836
Residuals	2396	30887	12.891			
Total	2479	$1.04 \cdot 10^5$				

[1]Ant numbers at the food source were counted every 30 seconds for 14.5 minutes; [2]*L. neglectus, L. niger* and *L. platythorax*; [3]comparisons of interest were pre-defined as contrasts (C1 - C4): *L. niger* vs. *L. neglectus*, *L. platythorax* vs. *L. neglectus*, *L. niger* vs. *L. platythorax* and *L. neglectus* vs. *L. neglectus*; the respective explorer species is given as subscript; [4]analysis of differences between trials within each competitor species, [5]and for pairwise comparisons of competitor species; [6]analysis of interactions among factors: analysis of the differences in the temporal change of ant numbers over all competitor species [7]and for pairwise comparisons of competitor species.

Table 3.11: Exploitative and interference competition (EIC) experiment comprising two parts carried out with species pairs of the species *L. neglectus*, *L. niger* and *L. platythorax*. Part 1: Only the explorer species was given access to the food source. Part 2: The explorer species and an additional competitor species were given access to the food source. Comparison of the number of ants when the species was the explorer species (part 1) with the number of ants when the species was the competitor species (part 2): PERMANOVA analyses with explorer ant numbers (part 1) and competitor ant numbers (part 2) of each species as response variable. The respective explorer species during part 2 is given as subscript. The PERMANOVA design took three factors (*time*, *group* and *trial number*) and the interaction between *time* and the pre-defined comparisons for each species (contrasts) into account. The table shows the degrees of freedom (df), sums of squares (SS), mean squares (MS), Pseudo-F values, P-values determined by permutations, and the numbers of unique permutations. P-values less than 0.05 are marked in bold.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P (perm)**	**Unique perms**
Time[1]	1	57127	57127	46.503	**< 0.001**	9813
Group[2]	6	$4.05 \cdot 10^5$	67486	47.069	**< 0.001**	9945
C1[3]: *L. neglectus* vs. *L. neglectus*$_{L.\ niger}$	1	$2.10 \cdot 10^5$	$2.10 \cdot 10^5$	637.11	**< 0.001**	9826
C2: *L. neglectus* vs. *L. neglectus*$_{L.\ platythorax}$	1	$2.02 \cdot 10^5$	$2.02 \cdot 10^5$	539.46	**< 0.001**	9833
C3: *L. niger* vs. *L. niger*$_{L.\ neglectus}$	1	74298	74298	222.08	**< 0.001**	9840
C4: *L. platythorax* vs. *L. platythorax*$_{L.\ neglectus}$	1	$1.05 \cdot 10^5$	$1.05 \cdot 10^5$	131.98	**< 0.001**	9836
Trial number (group)[4]	153	$2.49 \cdot 10^5$	1629.9	65.412	**< 0.001**	9800
Trial number (C1)[5]	58	66823	1152.1	49.884	**< 0.001**	9844
Trial number (C1)	58	76387	1317	54.511	**< 0.001**	9851
Trial number (C3)	38	49004	1289.6	78.952	**< 0.001**	9886
Trial number (C4)	38	$1.16 \cdot 10^5$	3039.5	70.113	**< 0.001**	9881
Time × group[6]	6	91104	15184	609.38	**< 0.001**	9938
Time × C1[7]	1	41013	41013	1775.8	**< 0.001**	9815
Time × C2	1	33846	33846	1400.8	**< 0.001**	9829
Time × C3	1	19706	19706	1206.4	**< 0.001**	9836
Time × C4	1	12151	12151	280.28	**< 0.001**	9840
Residuals	4713	$1.17 \cdot 10^5$	24.917			
Total	4879	$9.20 \cdot 10^5$				

[1]Ant numbers at the food source were counted every 30 seconds for 30 minutes; [2]*L. neglectus*, *L. niger*, *L. platythorax*, *L. neglectus*$_{L.\ niger}$, *L. neglectus*$_{L.\ platythorax}$, *L. niger*$_{L.\ neglectus}$ and *L. platythorax*$_{L.\ neglectus}$ (the respective explorer species is given as subscript); [3]comparisons of interest were pre-defined as contrasts (C1 - C4); [4]analysis of differences between trials within the groups, [5]and for pairwise comparisons of the two parts of the experiment for each species; [6]analysis of interactions among factors: analysis of the differences in the temporal change of ant numbers over all groups [7]and for pairwise comparisons of of the two parts of the experiment for each species.

Table 3.12: Exploitative and interference competition (EIC) experiment comprising two parts carried out with species pairs of the invasive species *L. neglectus*, and the two native species, *L. niger* and *L. platythorax*. Part 2: the explorer species and an additional competitor species were given access to the food source. Spearman rank correlations between the number of ants of the explorer species and the number of ants of the competitor species. The table shows the numbers of observations (N), Spearman's ρ-values, and P-values. P-values less than 0.05 are marked in bold.

Explorer species	**Competitor species**	**N**	ρ	P
L. neglectus	*L. niger*	620	-0.537	**< 0.001**
L. niger	*L. neglectus*	620	-0.382	**< 0.001**
L. neglectus	*L. platythorax*	620	-0.793	**< 0.001**
L. platythorax	*L. neglectus*	620	-0.488	**< 0.001**

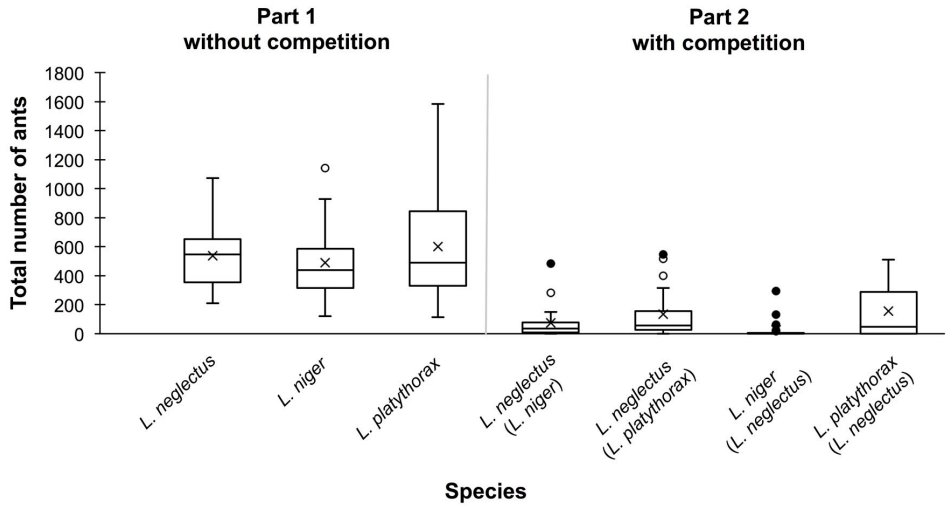


Figure 3.6: Total numbers of ants that were present at the food source during the exploitative and interference competition experiment. Part 1: explorer species in the absence of competition during the first part of the experiment. Part 2: Competitor species during the second part of the experiment. The respective explorer species that had occupied the food source is given in brackets. The figure shows box-and-whisker plots with first and third quartiles, median and whiskers (minimum and maximum value); mean (×); values that are more than 1.5 times the interquartile range (IQR) are displayed as outliers (○), values that are more than 3 IQR are displayed as extreme points (•).

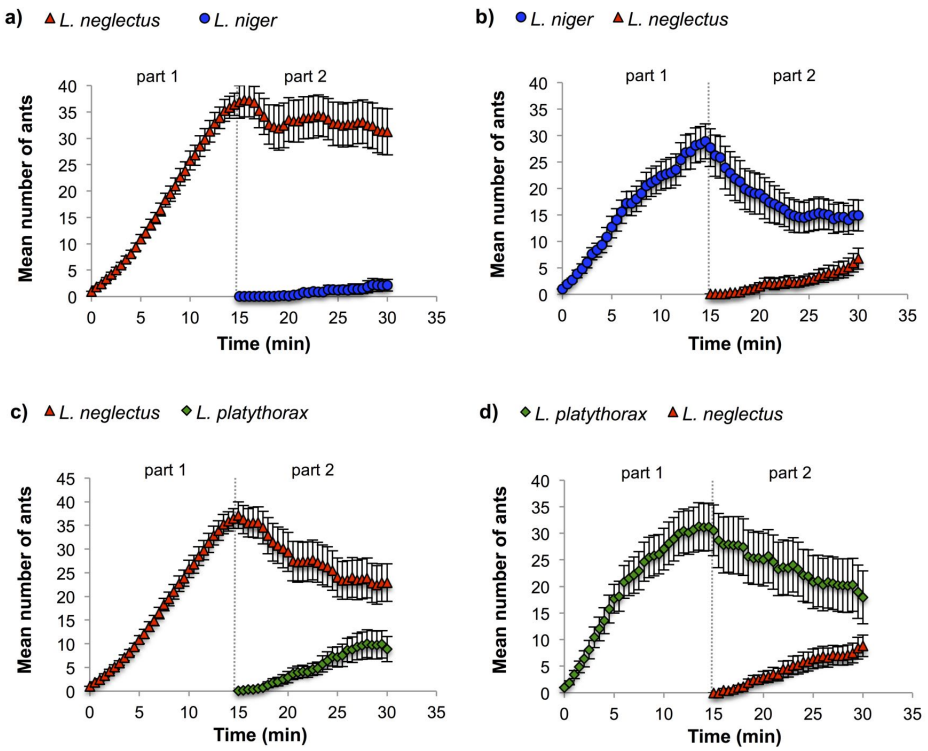


Figure 3.7: Exploitative and interference competition experiment. The figure shows mean numbers and standard errors of ants at the food source. Part 1: explorer species in the absence of competition during the first part of the experiment (minutes 0 – 14.5). Part 2: Competitor species during the second part of the experiment (minutes 15 – 30).

3.2.3 Results of the aggression experiments

Aggression indices were calculated for both species for each encounter considering peaceful or submissive, neutral and aggressive behaviors (see Equation 2.1 in Section 2.3.2). An aggression index of $AI = 1$ reflects exclusively peaceful or submissive behavior while an aggression index of $AI = -1$ reflects exclusively aggressive behavior.

3.2.3.1 Intraspecific aggression test with *F. fuscocinerea*

This section is based on Pohl et al. (2018)

To study intraspecific competition in *F. fuscocinerea*, aggression was investigated in aggression tests considering the distances of the locations the ants originated from.

Only non-aggressive behavior was observed in the *small distance approach* (Section 2.3.2.3) among different *F. fuscocinera* colony fragments of the study site and in the *large distance approach* (Section 2.3.2.3) among different populations originating from distant sites around Munich. Notably, the aggression indices did not increase with increasing distance between colonies (Spearman rank correlation (small distance): $N = 55, \rho = 0.032, P = 0.814$, (large distance): $N = 70, \rho = -0.220$, $P = 0.067$; Figure 3.8 A). Although both, aggression indices and antennation frequencies, significantly differed over all treatments (Table 3.13), this difference is mainly explained by the high aggressive behavior shown in allospecific encounters with *Formica* sp.: Aggression indices of receiver ants with intruder ants from different colony fragments and populations did not differ from those with nestmates (Table 3.14; Figure 3.8 B). In contrast, there were significant differences in aggression indices towards *Formica* sp., which was treated highly aggressively (Table 3.14; Figure 3.8 B).

The number of quick antennation interactions, i.e. one ant quickly touches the other ant with its antennas, did not increase with growing distances (Spearman rank correlation (small distance): $N = 55, \rho = -0.138, P = 0.315$, (large distance): $N = 70, \rho = -0.071, P = 0.559$). In the *small distance approach* the nestmate control and the treatment group with intruder ants from distant nests did not differ (Table 3.14). However, minor differences cannot be excluded, as the sample sizes of the nestmate controls were rather small. In the *large distance approach* the number of quick antennation interactions significantly differed between the nestmate control and the treatment group with intruder ants from distant populations (Table 3.14). However, the nestmate control showed the highest numbers of antennation interactions.

Table 3.13: Intraspecific competititon in *F. fuscocinerea*: Kruskal-Wallis comparison of the aggression indices and antennation frequencies of colony fragments of a population from Dachau (*small distance approach*) and of colony fragments of populations distributed around Munich (*large distance approach*). The table shows the numbers of observations (N), the test values (K) and two-sided Monte-Carlo *P*-values. *P*-values less than 0.05 are marked in bold.

Dimension	**Comparison**	**Group**	**N**	**K**	**two-sided *P***
small distance	Aggression index	Dachau pop.	47	32.655	**< 0.001**
		Control nestmate	8		
		Control *Formica* sp.	10		
	Antennation	Dachau pop.	47	15.554	**< 0.001**
		Control nestmate	8		
		Control *Formica* sp.	10		
large distance	Aggression index	Distant pop.	60	29.743	**< 0.001**
		Control nestmate	10		
		Control *Formica* sp.	10		
	Antennation	Distant pop.	60	28.811	**< 0.001**
		Control nestmate	10		
		Control *Formica* sp.	10		

Table 3.14: Intraspecific competititon in *F. fuscocinerea*: Post-hoc pairwise comparison with Dunn's procedure of the aggression indices and antennation frequencies of colony fragments of a population from Dachau (*small distance approach*) and of colony fragments of populations distributed around Munich (*large distance approach*). The table shows the pairwise differences ($W_{i,j}$) and the *P*-values. The significance level is Bonferroni corrected: *P*-values less than 0.017 are marked in bold.

Dimension	**Comparison**	**Groups**	**$W_{i,j}$**	**two-sided *P***
small distance	Aggression index	Dachau pop., Control nestmate	0.293	0.963
		Dachau pop., Control *Formica* sp.	-32.457	**< 0.001**
		Control nestmate, Control *Formica* sp.	-32.750	**< 0.001**
	Antennation	Dachau pop., Control nestmate	-7.737	0.281
		Dachau pop., Control *Formica* sp.	23.338	**< 0.001**
		Control nestmate, Control *Formica* sp.	31.075	**< 0.001**
large distance	Aggression index	Distant pop., Control nestmate	-5.133	0.492
		Distant pop., Control *Formica* sp.	-40.733	**< 0.001**
		Control nestmate, Control *Formica* sp.	-35.600	**< 0.001**
	Antennation	Distant pop., Control nestmate	-24.442	**0.002**
		Distant pop., Control *Formica* sp.	30.908	**< 0.001**
		Control nestmate, Control *Formica* sp.	55.350	**< 0.001**

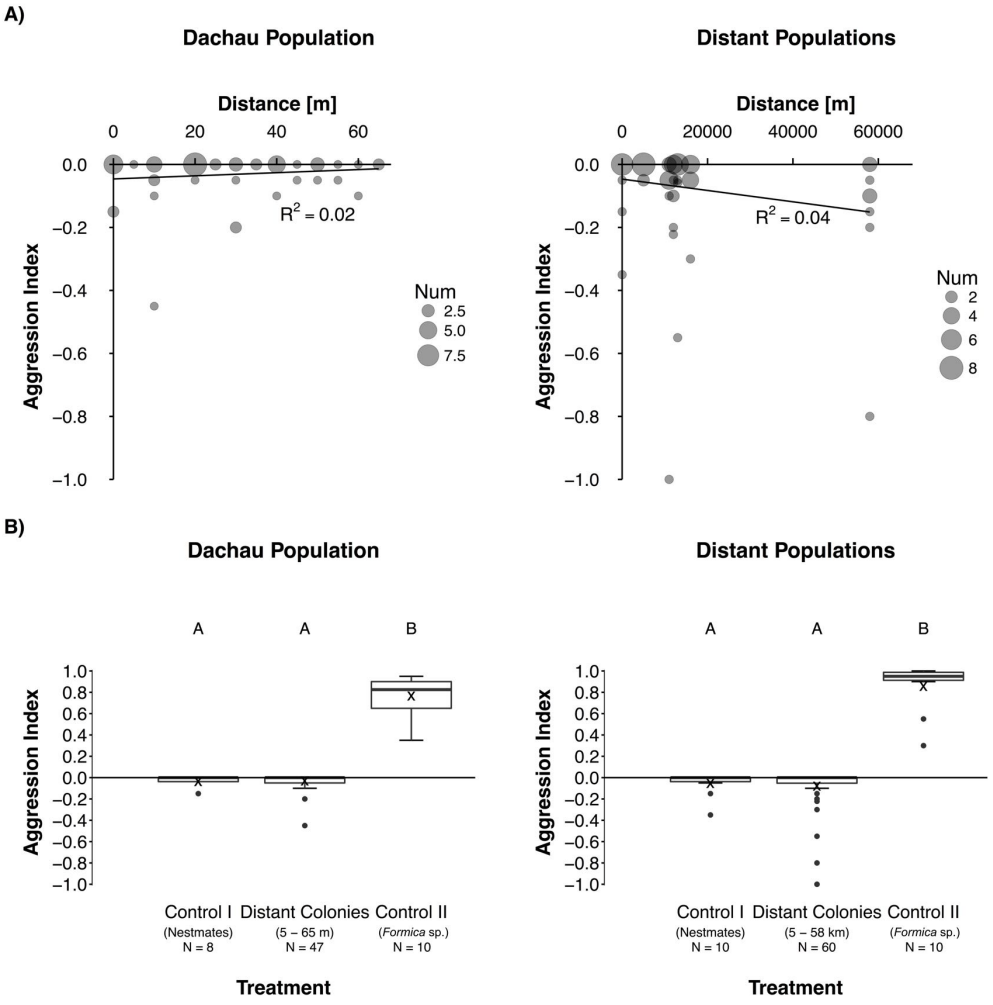


Figure 3.8: Intraspecific competition in *F. fuscocinerea* investigated through aggression tests within a population in Dachau (distance 0 - 65 m) and between distant populations in Southern Bavaria (distance 0 - 58000 m). Aggression indices were calculated considering aggressive, neutral and peaceful behavior. Positive aggression indices indicate predominantly aggressive behavior whereas negative aggression indices indicate mainly peaceful behavior. (A) Linear trend of the AIs with increasing distance between pairs of colony fragments. (B) Comparisons of the AIs of nestmate control (Control I), distant colonies and allospecific control (Control II) (box-and-whisker plots with first and third quartiles, median and whiskers (minimum and maximum value); mean (×); values that are more than 1.5 times the interquartile range (IQR) are displayed as outliers (●)). Different upper case letters denote significant differences (Kruskal-Wallis pairwise comparisons, $P \leq 0.001$). Source: Pohl et al. (2018)

3.2.3.2 One-on-one aggression test with *L. neglectus, L. niger* and *L. platythorax*

The three species *L. neglectus, L. niger* and *L. platythorax* differed in their aggression indices when they were in one-on-one encounters (Kruskal-Wallis test, $K = 29.093$, Monte-Carlo $P < 0.001$, for detailed numbers of N see Table A.7 in Appendix A.1.5): The invasive *Lasius neglectus* showed more aggressive than neutral or peaceful behavior when being confronted with native *Lasius* ants (Figure 3.9). There was no difference in its aggressiveness whether it encountered *L. niger* or *L. platythorax* (see effect of $L.\ neglectus_{L.\ niger}$ vs. $L.\ neglectus_{L.\ platythorax}$ in Table 3.15; Figure 3.9). While *L. platythorax* responded *L. neglectus* with the same amount of aggressive behavior, *L. niger* behaved more cautiously in encounters with *L. neglectus* (see effects of $L.\ neglectus_{L.\ platythorax}$ vs. $L.\ platythorax_{L.\ neglectus}$ and $L.\ neglectus_{L.\ niger}$ vs. $L.\ niger_{L.\ neglectus}$ and $L.\ niger_{L.\ neglectus}$ vs. $L.\ platythorax_{L.\ neglectus}$ in Table 3.15; Figure 3.9). During the time span of three minutes there was neither aggressive nor peaceful behavior detectable in the nestmate control trials (Figure 3.9).

3.2.3.3 Aggression test with groups of ants of *L. neglectus, L. niger* and *L. platythorax*

The explorer species, i.e. the species that had sole access to the food source during the first 15 minutes of the EIC experiment, all behaved highly aggressively when a competitor species arrived. There was no difference in the aggressive behavior of *L. neglectus, L. niger* and *L. platythorax* (PERMANOVA: Pseudo-$F_{3,45} = 0.788$, $P = 0.496$; Table 3.16 a ; Figure 3.10 A). Arriving as a competitor at an already occupied food source the three species differed in their behavior (PERMANOVA: Pseudo-$F_{3,45} = 25.393$, $P < 0.001$; Table 3.16 b; Figure 3.10 B): As a competitor the invasive *L. neglectus* was still highly aggressive against both native species (see effects of $L.\ neglectus_{L.\ niger}$ and $L.\ neglectus_{L.\ platythorax}$ in Table 3.16 b; see boxplots for *L. neglectus(L. niger)* and *L. neglectus(L. platythorax)* in Figure 3.10 B). In contrast, *L. niger* and *L. platythorax* were rather submissive when arriving at food sources that were already occupied by *L. neglectus* (see effects of $L.\ niger_{L.\ neglectus}$ and $L.\ platythorax_{L.\ neglectus}$ in Table 3.16 b; see boxplots for *L. niger(L. neglectus)* and *L. platythorax(L. neglectus)* in Figure 3.10 B). *Lasius niger* behaved even more defensively than *L. platythorax*.

Especially the native *Lasius* species showed different degrees of aggressive behavior according to their competitive position. Both, *L. niger* and *L. platythorax*, behaved apparently more aggressively when defending their own discovered and occupied food source than when arriving as a new competitor at an already occupied food source (*L. niger*: Mann-Whitney-U-Test, $N_{explorer}$ =10, $N_{competitor}$ = 15, $U = 147.5$, Monte-Carlo $P < 0.001$; *L. platythorax*: Mann-Whitney-U-Test, $N_{explorer}$ =9, $N_{competitor}$ = 15, $U = 125.0$, Monte-Carlo $P < 0.001$; Figure 3.10). Interestingly, the invasive *L. neglectus* behaved even more aggressively towards *L. niger* when *L. neglectus* arrived as a new competitor than when it had to defend the food source as an explorer species (*L. neglectus*: Mann-Whitney-U-Test, $N_{explorer}$ = 15, $N_{competitor}$ = 10, $U = 37.0$, Monte-Carlo $P = 0.026$; Figure 3.10). In competition with *L. platythorax* the invasive *L. neglectus* did not change its aggressive behavior (*L. neglectus*: Mann-Whitney-U-Test, $N_{explorer}$ = 15, $N_{competitor}$ = 9, $U = 58.0$, Monte-Carlo $P = 0.573$; Figure 3.10).

Table 3.15: One-on-one aggression tests with the invasive species *L. neglectus* and the native species, *L. niger* and *L. platythorax*. Kruskal-Wallis pairwise comparison with Dunn's procedure of the aggression indices of the species considering their opponents (shown as subscripts). The table shows the pairwise differences ($W_{i,j}$) and the Monte-Carlo *P*-values. The significance level is Bonferroni corrected: *P*-values less than 0.003 are marked in bold.

Comparison			$\mathbf{W_{i,j}}$	*P*
L. neglectus$_{L.\ niger}$	vs.	*L. neglectus*$_{L.\ platythorax}$	0.100	0.991
L. neglectus$_{L.\ niger}$	vs.	*L. niger*$_{L.\ neglectus}$	40.033	**< 0.001**
L. neglectus$_{L.\ niger}$	vs.	*L. platythorax*$_{L.\ neglectus}$	12.933	0.155
L. neglectus$_{L.\ niger}$	vs.	*L. neglectus*$_{\text{nestmate control}}$	22.867	0.018
L. neglectus$_{L.\ niger}$	vs.	*L. niger*$_{\text{nestmate control}}$	22.867	0.018
L. neglectus$_{L.\ niger}$	vs.	*L. platythorax*$_{\text{nestmate control}}$	22.867	0.018
L. neglectus$_{L.\ platythorax}$	vs.	*L. platythorax*$_{L.\ neglectus}$	12.833	0.158
L. neglectus$_{L.\ platythorax}$	vs.	*L. niger*$_{L.\ neglectus}$	39.933	**< 0.001**
L. neglectus$_{L.\ platythorax}$	vs.	*L. neglectus*$_{\text{nestmate control}}$	22.767	0.018
L. neglectus$_{L.\ platythorax}$	vs.	*L. platythorax*$_{\text{nestmate control}}$	22.767	0.018
L. neglectus$_{L.\ platythorax}$	vs.	*L. niger*$_{\text{nestmate control}}$	22.767	0.018
L. neglectus$_{\text{nestmate control}}$	vs.	*L. niger*$_{L.\ neglectus}$	17.167	0.075
L. neglectus$_{\text{nestmate control}}$	vs.	*L. platythorax*$_{L.\ neglectus}$	-9.933	0.303
L. neglectus$_{\text{nestmate control}}$	vs.	*L. niger*$_{\text{nestmate control}}$	0	1
L. neglectus$_{\text{nestmate control}}$	vs.	*L. platythorax*$_{\text{nestmate control}}$	0	1
L. niger$_{L.\ neglectus}$	vs.	*L. platythorax*$_{L.\ neglectus}$	-27.100	0.003
L. niger$_{L.\ neglectus}$	vs.	*L. niger*$_{\text{nestmate control}}$	-17.167	0.075
L. niger$_{L.\ neglectus}$	vs.	*L. platythorax*$_{\text{nestmate control}}$	-17.167	0.075
L. niger$_{\text{nestmate control}}$	vs.	*L. platythorax*$_{L.\ neglectus}$	-9.933	0.303
L. niger$_{\text{nestmate control}}$	vs.	*L. platythorax*$_{\text{nestmate control}}$	0	1
L. platythorax$_{L.\ neglectus}$	vs.	*L. platythorax*$_{\text{nestmate control}}$	9.333	0.303

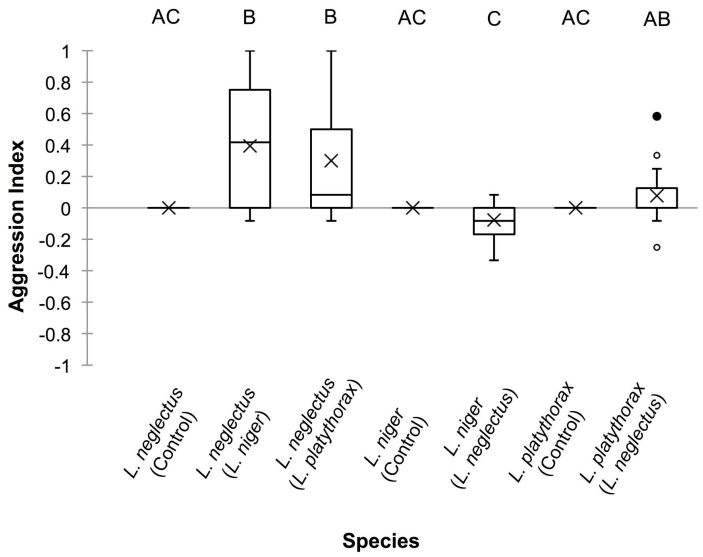


Figure 3.9: Interspecific competition in *Lasius* investigated in one-on-one aggression tests with *L. neglectus*, *L. niger* and *L. platythorax*. Aggression indices were calculated considering aggressive, neutral and peaceful behavior. Positive aggression indices indicate predominantly aggressive behavior whereas negative aggression indices indicate mainly peaceful behavior. Comparison of the aggression indices of the three species and of the nestmate controls. The respective opponent is given in brackets. The figure shows box-and-whisker plots with first and third quartiles, median and whiskers (minimum and maximum value); mean (×); values that are more than 1.5 times the interquartile range (IQR) are displayed as outliers (∘), values that are more than 3 IQR are displayed as extreme points (•). Different upper case letters denote significant differences (Kruskal-Wallis pairwise comparisons, $P \leq 0.05$ without Bonferroni corrections).

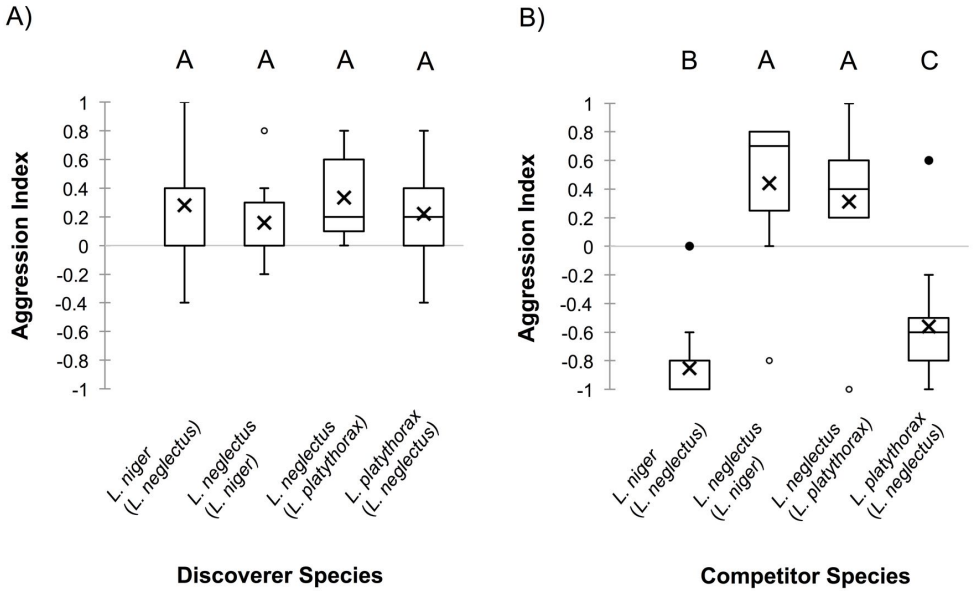


Figure 3.10: Interspecific competition in *Lasius* investigated during group encounters with *L. neglectus*, *L. niger* and *L. platythorax*. Aggression indices were calculated considering aggressive, neutral and submissive behavior. Aggression indices were calculated considering aggressive, neutral and peaceful behavior. Positive aggression indices indicate predominantly aggressive behavior whereas negative aggression indices indicate mainly submissive behavior. Comparison of the aggression indices of the three species. The respective opponent (A) competitor species B) explorer species) is given in brackets. The figure shows box-and-whisker plots with first and third quartiles, median and whiskers (minimum and maximum value); mean (×); values that are more than 1.5 times the interquartile range (IQR) are displayed as outliers (○), values that are more than 3 IQR are displayed as extreme points (•). Different upper case letters denote significant differences (PERMANOVA, pairwise comparisons, $P \leq 0.05$).

Table 3.16: Aggression test with groups of ants of the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*. PERMANOVA post-hoc pairwise comparisons with the aggression indices of a) the explorer and b) the competitor species as response variable. The respective a) competitor or b) explorer species is given as subscript. The table shows the test statistics (t), *P*-values determined by permutations, and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

a) Comparison of explorer species			**t**	***P* (perm)**	**Unique perms**
$L.\ neglectus_{L.\ niger}$	vs.	$L.\ niger_{L.\ neglectus}$	0.935	0.383	27
$L.\ neglectus_{L.\ niger}$	vs.	$L.\ neglectus_{L.\ platythorax}$	1.682	0.128	14
$L.\ neglectus_{L.\ niger}$	vs.	$L.\ platythorax_{L.\ neglectus}$	0.503	0.676	22
$L.\ niger_{L.\ neglectus}$	vs.	$L.\ neglectus_{L.\ platythorax}$	0.381	0.725	30
$L.\ niger_{L.\ neglectus}$	vs.	$L.\ platythorax_{L.\ neglectus}$	0.337	0.801	27
$L.\ neglectus_{L.\ platythorax}$	vs.	$L.\ platythorax_{L.\ neglectus}$	0.810	0.455	26
b) Comparison of competitor species			**t**	***P* (perm)**	**Unique perms**
$L.\ neglectus_{L.\ niger}$	vs.	$L.\ niger_{L.\ neglectus}$	7.984	**< 0.001**	63
$L.\ neglectus_{L.\ niger}$	vs.	$L.\ neglectus_{L.\ platythorax}$	0.520	0.61	33
$L.\ neglectus_{L.\ niger}$	vs.	$L.\ platythorax_{L.\ neglectus}$	5.318	**< 0.001**	31
$L.\ niger_{L.\ neglectus}$	vs.	$L.\ neglectus_{L.\ platythorax}$	6.784	**< 0.001**	54
$L.\ niger_{L.\ neglectus}$	vs.	$L.\ platythorax_{L.\ neglectus}$	2.249	**0.034**	18
$L.\ neglectus_{L.\ platythorax}$	vs.	$L.\ platythorax_{L.\ neglectus}$	4.376	**< 0.001**	27

3.3 Pheromone trail experiments

Pheromone trail communication was studied in the invasive *L. neglectus* and the native species, *L. niger* and *L. platythorax* (Section 2.4). The pheromone sources hindgut, mandibular gland, poison gland and Dufour's gland were tested in four different gland trail experiments. In three experiments gland solvents were applied to paper bridges as artificial gland trails. In the fourth experiment the dissected glands were provided directly to the ants. In the direction-by-choice experiment ants could choose between two legs of a Y-bifurcation. One leg was treated with the gland solvent the other leg was treated with the control solvent dichloromethane (DCM). In the accuracy-in-trail-following experiment the artificial gland trail or the DCM control trail was applied as an S-shaped curve. In the alternative-trail-branch experiment the ants could choose to follow either their natural trail or to turn onto a branch with an artificial gland trail or DCM control trail. The single-point-source experiment tested differences in behavioral reactions of the ants to concentrated gland substances which were provided as a point sources next to natural gland trails.

3.3.1 Direction-by-choice experiment

Almost all glands attracted more ants than the DCM control trails except the poison gland in *L. neglectus* and the Dufour's gland in *L. platythorax* (Table 3.17, Figure 3.11). Hence, the glands acted as attractants rather than repellents to the ants. The most attractive gland trail for all three species was the hindgut trail (Table 3.18, Figure 3.11; for the main test see Table A.10 in the Appendix). The species did not differ in their preference for hindgut trails (Table 3.19, Figure 3.11). In case of the native species, *L. niger* and *L. platythorax*, the mandibular gland trail was the second most attractive gland trail (Table 3.18, Figure 3.11). The invasive *L. neglectus* was less attracted by the mandibular gland compared to the native species (Table 3.18, Figure 3.11): The attractiveness of the mandibular gland trails did not differ from Dufour's gland trails in *L. neglectus* (Table 3.18). Poison gland trails attracted equal number of ants to Dufour's gland trails, in *L. niger* and in *L. platythorax* (Table 3.18, Figure 3.11). For the invasive species the poison gland was the least attractive gland trail: The number of *L. neglectus* ants on the poison gland trail did not differ from the DCM control trail (Table 3.17, Figure 3.11).

Trail specificity in *Lasius* ants In general *L. neglectus*, *L. niger* and *L. platythorax* followed specifically conspecific hindgut trails when having the choice between a conspecific and an allospecific hindgut trail branch (Table 3.20, Figure 3.12).

Table 3.17: Direction-by-choice experiment with the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*: Wilcoxon signed-rank test comparing the numbers of ants following the artificial gland trail with the numbers of ants following the control trail on a Y-bifurcation. The table shows the sample sizes (N), the test statistics (W) and the two-sided Monte-Carlo P-values. P-values less than 0.05 are marked in bold.

	L. neglectus			***L. niger***			***L. platythorax***		
Comparison	N	W	two-sided P	N	W	two-sided P	N	W	two-sided P
H[1], C[2]	22	253.0	**< 0.001**	21	231.0	**< 0.001**	21	231.0	**< 0.001**
M[3], C	20	168.0	**< 0.001**	21	210.0	**< 0.001**	21	210.0	**< 0.001**
P[4], C	25	83.0	0.714	21	114.5	**0.010**	21	175.5	**0.005**
D[5], C	20	166.5	**< 0.001**	21	190.0	**< 0.001**	21	103.0	0.706

[1]Hindgut (H), [2]DCM control, [3]mandibular gland (M), [4]poison gland (P), [5]Dufour's gland (D)

Table 3.18: Direction-by-choice experiment with the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*: PERMANOVA post-hoc pairwise comparisons of the glands for each species. Analyzed is the number of ants that chose the gland trails on a Y-bifurcation. The table shows the test statistics (t), *P*-values determined by permutations, and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

Comparison	***L. neglectus***			***L. niger***			***L. platythorax***		
	t	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**
H[1], M[2]	7.9578	**< 0.001**	38	2.4007	**0.022**	285	4.358	**< 0.001**	16
H, P[3]	10.336	**< 0.001**	60	6.2605	**< 0.001**	1473	6.4613	**< 0.001**	25
H, D[4]	7.4064	**< 0.001**	40	6.2075	**< 0.001**	1071	9.0783	**< 0.001**	32
M, P	3.5919	**0.001**	42	4.0224	**< 0.001**	24	2.9091	**0.008**	23
M, D	0.12188	1	16	3.6683	**< 0.001**	23	5.1908	**< 0.001**	27
P, D	3.3412	**0.002**	42	0.60423	0.604	23	1.9655	0.065	25

[1]Hindgut (H), [2]mandibular gland (M), [3]poison gland (P), [4]Dufour's gland (D)

Table 3.19: Direction-by-choice experiment with the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*: PERMANOVA post-hoc pairwise comparisons of the species for each gland type. Analyzed is the number of ants that chose the gland trails on a Y-bifurcation. The table shows the test statistics (t), *P*-values determined by permutations, and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

Gland	***L. neglectus, L. niger***			***L. neglectus, L. platythorax***			***L. niger, L. platythorax***		
	t	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**
H[1]	0.76662	0.466	160	1.1338	0.303	17	0.37719	0.744	43
M[2]	4.5071	**< 0.001**	38	3.1291	**0.004**	35	1.294	0.246	17
P[3]	2.1148	**0.045**	47	2.3198	**0.027**	50	0.14396	0.942	25
D[4]	2.7415E-2	1	38	2.7643	**0.012**	43	2.5236	**0.021**	25

[1]Hindgut (H), [2]mandibular gland (M), [3]poison gland (P), [4]Dufour's gland (D)

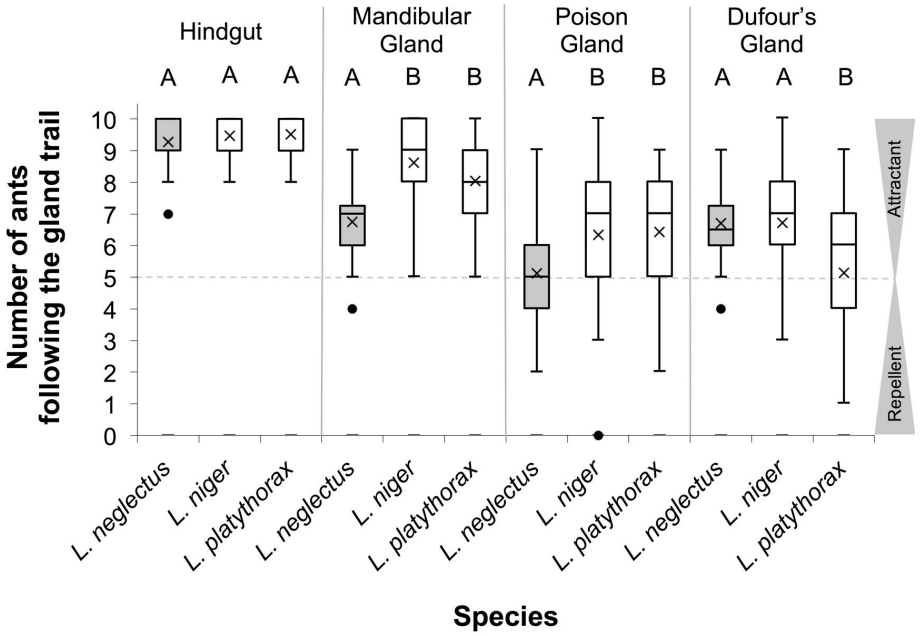


Figure 3.11: Direction-by-choice experiment with the invasive *L. neglectus* (boxes shaded in grey) and the native, *L. niger* and *L. platythorax*. Displayed is the number of ants choosing the arm of the Y-bifurcation with the artificial gland trail. Box-and-whisker plots with first and third quartiles, median and whiskers (minimum and maximum value); mean (×); values that are more than 1.5 times the interquartile range (IQR) are displayed as outliers (●). Different upper case letters denote significant differences between the species within a gland type (PERMANOVA post-hoc pairwise comparisons, $P < 0.05$).

Table 3.20: Trail specificity in *Lasius* ants: Wilcoxon signed-rank test comparing the numbers of ants following the conspecific hindgut trail with the numbers of ants following the allospecific hindgut trail on a Y-bifurcation. The table shows the sample sizes (N), the test statistics (W) and the two-sided Monte-Carlo P-values. P-values less than 0.05 are marked in bold.

Testspecies	**Allospecific hindgut source**	N	W	**two-sided** P
L. neglectus	*L. niger*	20	210	**< 0.001**
	L. platythorax	20	210	**< 0.001**
L. niger	*L. neglectus*	20	210	**< 0.001**
	L. platythorax	20	210	**< 0.001**
L. platythorax	*L. neglectus*	20	210	**< 0.001**
	L. niger	20	210	**< 0.001**

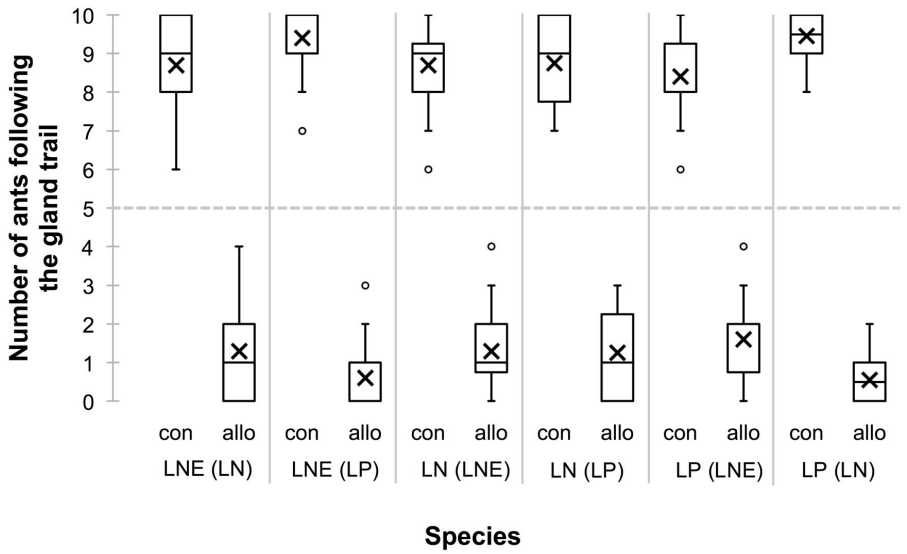


Figure 3.12: Species-specificity of hindgut trails in *Lasius*: Direction-by-choice experiment with the invasive *L. neglectus* (LNE) and the native, *L. niger* (LN) and *L. platythorax* (LP). Displayed is the number of ants that followed the branch with the conspecific gland trail (con) and the number of ants that followed the allospecific gland trail (allo). The respective source for the allospecific trail is given in brackets. Box-and-whisker plots with first and third quartiles, median and whiskers (minimum and maximum value); mean (×); values that are more than 1.5 times the interquartile range (IQR) are displayed as outliers (∘).

3.3.2 Accuracy-in-trail-following experiment

The invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*, followed artificial gland trails with varying accuracy depending on the offered gland type (Table 3.21; for the main tests see Table A.13 in Appendix A.1.7.2): All three species followed most accurately the hindgut trail over the entire length at least compared to poison gland, Dufour's gland and both control trails (see effects of (*H, P*), (*H, D*), (*H, C*) and (*H, PC*) in Table 3.21; Figure 3.13). In the native *L. niger* the mandibular gland also induced an obvious trail following which differed from the remaining glands at a length of three quarters of the trail (see effects of (*M, P*), (*M, D*), (*M, C*) and (*M, PC*) at trail length 3/4 in Table 3.21; Figure 3.13). The longer the complex artificial trail was, the fewer ants followed: For hindgut, poison gland, Dufour's gland, the pencil control, the mandibular gland (*L. neglectus*) and the DCM control (*L. neglectus*, *L. niger*), the number of ants that followed the entire trail was significantly lower than the number of ants that followed the first quarter of the trail (Table 3.22). Except in the native species, *L. niger* and *L. platythorax*, where similar number of ants followed the first quarter and the entire trail length on mandibular trails (see effect of (*M*| *1/4,1*) in Table 3.22). The comparison of the species shows, that there were only minor differences in the number of ants following the artificial trails. Most interestingly, significantly less ants of the invasive *L. neglectus* followed the mandibular gland than ants of the native species (see effects of *M* of the comparisons (*L. neglectus*, *L. niger*) and (*L. neglectus*, *L. platythorax*) in Table 3.23; Figure 3.13; for the main test see Table A.15 in Appendix A.1.7.2). In contrast, equal numbers of native ants followed the mandibular gland trails (see effects of *M* of the comparison (*L. niger*, *L. platythorax*) in Table 3.23; Figure 3.13).

Table 3.21: Accuracy-in-trail-following experiment with the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*: PERMANOVA post-hoc pairwise comparisons of the glands for each species. Analyzed is the number of ants that followed an artificial S-shaped gland trail which is divided into four sections. The table shows the test statistics (t), *P*-values determined by permutations, and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

	L. neglectus			***L. niger***			***L. platythorax***		
Comparison	**t**	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**
H[1], M[2]	2.3058	**0.006**	9953	1.7018	0.161	9974	1.7283	0.054	9956
H, M | 1/4[7]	2.8234	**0.002**	9954	1.9248	0.081	9967	1.5244	0.117	9941
H, M | 1/2[8]	2.6407	**0.004**	9963	1.9894	0.077	9962	1.6237	0.095	9958
H, M | 3/4[9]	2.0923	**0.036**	9964	1.9012	0.075	9965	1.8005	0.063	9969
H, M | 1[10]	4.537	**< 0.001**	9956	1.5228	0.170	9955	1.9643	**0.039**	9954
H, P[3]	3.4654	**< 0.001**	9962	2.8838	**0.002**	9976	2.2918	**0.004**	9950
H, P | 1/4	3.8221	**< 0.001**	9960	2.575	**0.027**	9962	3.1007	**0.005**	9966
H, P | 1/2	4.072	**< 0.001**	9957	3.186	**0.009**	9964	2.1936	**0.018**	9940
H, P | 3/4	6.4051	**< 0.001**	9961	3.7072	**0.002**	9957	2.9797	**0.002**	9954
H, P | 1	6.4783	**< 0.001**	9965	3.6461	**0.001**	9960	2.6011	**0.006**	9947
H, D[4]	3.9171	**< 0.001**	9961	2.3089	**0.016**	9962	2.3384	**0.008**	9957
H, D | 1/4	3.5103	**< 0.001**	9953	1.9558	0.090	9971	1.8772	0.087	9963
H, D | 1/2	6.1127	**< 0.001**	9959	2.436	**0.033**	9955	2.1137	0.055	9947
H, D | 3/4	7.4644	**< 0.001**	9955	3.2431	**0.008**	9968	3.6102	**0.004**	9958
H, D | 1	6.9508	**< 0.001**	9964	2.575	**0.014**	9968	4.4346	**0.001**	9958
H, C[5]	2.6956	**0.001**	9960	4.0933	**< 0.001**	9961	4.2366	**< 0.001**	9958
H, C | 1/4	2.9142	**0.004**	9957	3.9631	**0.002**	9959	2.1297	**0.037**	9950
H, C | 1/2	2.0567	**0.037**	9955	6.6324	**< 0.001**	9961	3.8091	**0.002**	9957
H, C | 3/4	6.3042	**< 0.001**	9961	6.8707	**< 0.001**	9963	8.5315	**< 0.001**	9954
H, C | 1	7.8542	**< 0.001**	9957	5.6272	**< 0.001**	9958	7.1329	**< 0.001**	9953
H, PC[6]	4.5023	**< 0.001**	9962	3.8157	**< 0.001**	9963	4.2683	**< 0.001**	9963
H, PC | 1/4	5.4286	**< 0.001**	9958	2.0558	0.066	9948	3.1411	**0.005**	9963
H, PC | 1/2	5.5256	**< 0.001**	9952	5.7285	**< 0.001**	9966	5.1005	**0.002**	9956
H, PC | 3/4	8.5728	**< 0.001**	9970	6.6902	**< 0.001**	9957	7.0445	**< 0.001**	9956
H, PC | 1	7.921	**< 0.001**	9960	6.1073	**< 0.001**	9966	6.735	**< 0.001**	9963
M, P	0.5996	0.998	9946	2.2462	**0.022**	9975	1.1795	0.397	9928
M, P | 1/4	0.84171	0.655	9960	1.5619	0.189	9968	1.6125	0.111	9955
M, P | 1/2	1.0136	0.475	9951	2.7751	**0.010**	9971	1.2477	0.244	9955
M, P | 3/4	0.7251	0.767	9950	3.0476	**0.011**	9967	1.5646	0.131	9950
M, P | 1	1.018	0.464	9955	2.5844	**0.023**	9962	1.1109	0.356	9943
M, D	0.77755	0.952	9962	1.4938	0.247	9960	1.5796	0.119	9958
M, D | 1/4	1.0385	0.439	9954	1.0031	0.459	9958	1.4514	0.171	9967
M, D | 1/2	1.6064	0.120	9955	1.701	0.116	9960	1.4699	0.163	9970
M, D | 3/4	0.88591	0.597	9957	2.3085	**0.038**	9958	2.3009	**0.041**	9964
M, D | 1	1.0364	0.456	9950	1.6853	0.091	9947	2.1271	0.051	9964
M, C	0.53336	0.999	9960	3.2972	**0.001**	9961	2.2163	**0.011**	9964
M, C | 1/4	0.77008	0.749	9948	2.7891	**0.015**	9960	1.5219	0.129	9967
M, C | 1/2	0.62971	0.869	9958	6.4344	**< 0.001**	9971	2.0033	0.055	9946
M, C | 3/4	0.72979	0.768	9955	5.2632	**< 0.001**	9957	3.0468	**0.005**	9951
M, C | 1	1.2929	0.253	9952	3.6929	**0.002**	9956	2.2919	**0.021**	9963

Continued on next page

Table 3.21 Continued from previous page

Comparison	*L. neglectus*			*L. niger*			*L. platythorax*		
	t	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**
M, PC	0.99239	0.733	9941	3.0228	**0.003**	9976	2.9445	**0.001**	9952
M, PC | 1/4	1.4988	0.127	9930	1.5697	0.170	9953	2.9857	**0.005**	9946
M, PC | 1/2	1.6728	0.104	9945	5.1336	**< 0.001**	9966	3.47	**0.007**	9960
M, PC | 3/4	0.98343	0.499	9955	5.0751	**< 0.001**	9952	3.7758	**0.007**	9969
M, PC | 1	1.2998	0.253	9963	3.9763	**0.002**	9958	3.105	**0.013**	9966
P, D	0.52067	1	9951	1.1834	0.558	9965	0.91968	0.815	9930
P, D | 1/4	1.1779	0.303	9954	0.84542	0.634	9956	1.0836	0.381	9956
P, D | 1/2	1.0693	0.412	9961	1.3133	0.262	9955	0.75637	0.769	9959
P, D | 3/4	1.0599	0.425	9964	1.842	0.101	9964	1.2238	0.288	9959
P, D | 1	1.053	0.440	9954	1,479	0.192	9966	1.2865	0.254	9953
P, C	0.39141	1	9950	1.2962	0.442	9966	1.4228	0.169	9959
P, C | 1/4	0.74855	0.773	9955	0.93058	0.555	9955	1.3098	0.195	9948
P, C | 1/2	0.48618	0.954	9963	2.7999	**0.013**	9958	1.2745	0.246	9961
P, C | 3/4	0.63713	0.883	9964	1.9085	0.056	9954	1.7048	0.099	9954
P, C | 1	0.90916	0.554	9966	1.9201	0.065	9972	1.3447	0.231	9956
P, PC	0.8222	0.927	9942	1.1272	0.622	9964	2.026	**0.021**	9965
P, PC | 1/4	2.2212	**0.011**	9946	0.69219	0.793	9962	2.5007	**0.009**	9960
P, PC | 1/2	1.4876	0.172	9950	1.9767	0.063	9957	2.3148	**0.026**	9946
P, PC | 3/4	1.1324	0.362	9960	1.6218	0.112	9951	2.2509	**0.035**	9947
P, PC | 1	0.90766	0.567	9964	1.9949	0.053	9955	1.8564	0.085	9938
D, C	0.47135	1	9933	1.83	0.123	9959	0.68369	0.983	9960
D, C | 1/4	0.85542	0.661	9954	1.5361	0.193	9961	0.43684	0.995	9946
D, C | 1/2	0.74451	0.737	9951	3.047	**0.016**	9956	0.90084	0.581	9951
D, C | 3/4	1.0423	0.436	9944	3.1292	**0.011**	9967	1.0223	0.456	9955
D, C | 1	1.1218	0.38	9953	1.8454	0.098	9973	1.0226	0.455	9956
D, PC	0.58753	1	9949	1.6867	0.18	9970	0.77832	0.927	9941
D, PC | 1/4	1.9216	**0.027**	9956	1.0924	0.413	9960	0.80858	0.669	9968
D, PC | 1/2	0.73516	0.761	9952	2.2356	0.066	9967	1.2588	0.282	9957
D, PC | 3/4	1.121	0.374	9961	3.4155	**0.008**	9975	0.85648	0.635	9956
D, PC | 1	1.121	0.385	9960	2.0127	0.071	9973	1.0035	0.471	9964
C, PC	0.69586	0.978	9953	0.4942	1	9966	0.54145	1	9941
C, PC | 1/4	1.6771	0.088	9965	0.5671	0.904	9953	0.67529	0.834	9963
C, PC | 1/2	0.83191	0.651	9954	1.8039	0.127	9976	0.54775	0.89	9961
C, PC | 3/4	1.3583	0.220	9960	0.53285	0.833	9968	0.98995	0.483	9953
C, PC | 1	*[11]			0.92828	0.532	9970	*		

[1]Hindgut, [2]mandibular gland, [3]poison gland, [4]Dufour's gland, [5]DCM control, [6]pencil control, [7]comparison of the numbers of ants that followed the first quarter of the gland trail lenght, [8]comparison of the numbers of ants that followed the first half of the gland trail lenght, [9]comparison of the numbers of ants that followed three quarters of the gland trail length, [10]comparison of the numbers of ants that followed the entire gland trail, [11]denominator is 0

Table 3.22: Accuracy-in-trail-following experiment with the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*: PERMANOVA post-hoc pairwise comparisons for each gland and for each species. Compared are the numbers of ants that followed the first quarter of an artificial S-shaped gland trail with the numbers of ants that followed the entire length of the gland trail. The table shows the test statistics (t), *P*-values determined by permutations, and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

	L. neglectus			***L. niger***			***L. platythorax***		
Comparison	**t**	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**
H[1] | 1/4[7], 1[8]	1.7712	**0.042**	9927	2.8453	**0.004**	9881	3.1917	**0.006**	9906
M[2] | 1/4, 1	6.3596	**< 0.001**	9907	1.3638	0.078	9867	1.5079	0.096	9917
P[3] | 1/4, 1	5.1552	**< 0.001**	9923	3.5732	**0.002**	9874	1.9622	**0.043**	9911
D[4] | 1/4, 1	3.8875	**< 0.001**	9918	1.8911	**0.022**	9861	2.9969	**0.016**	9916
C[5] | 1/4, 1	3.9964	**0.001**	9896	3.2499	**0.004**	9844	1.6162	0.087	9900
PC[6] | 1/4, 1	5.9103	**< 0.001**	9911	1.9296	**0.030**	9875	2.9495	**0.019**	9920

[1]Hindgut, [2]mandibular gland, [3]poison gland, [4]Dufour's gland, [5]DCM control, [6]pencil control, [7]comparison of the numbers of ants that followed the first quarter of the gland trail lenght, [8]with the numbers of ants that followed the entire length of the gland trail

Table 3.23: Accuracy-in-trail-following experiment with the invasive *L. neglectus* and the native *L. niger* and *L. platythorax*: PERMANOVA post-hoc pairwise comparisons of the species for each gland type. Analyzed is the number of ants that followed an artificial S-shaped gland trail which is divided into four sections. The table shows the test statistics (t), *P*-values determined by permutations, and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

	L. neglectus, L. niger			***L. neglectus, L. platythorax***			***L. niger, L. platythorax***		
Groups	**t**	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**
H[1] | 1/4[7]	0.67553	0.528	9925	1.1274	0.237	9934	0.69728	0.694	9934
H | 1/2[8]	1.1173	0.253	9938	1.5742	0.087	9940	0.81193	0.491	9939
H | 3/4[9]	1.68	0.081	9943	1.8701	**0.041**	9935	0.47767	0.798	9951
H | 1[10]	1.2744	0.195	9947	2.2984	**0.011**	9953	0.96508	0.357	9940
M[2] | 1/4	2.5886	**< 0.001**	9940	1.8729	**0.032**	9946	0.865	0.478	9940
M | 1/2	3.119	**< 0.001**	9935	2.2036	**0.016**	9942	0.8409	0.523	9945
M | 3/4	4.3751	**< 0.001**	9942	3.3881	**< 0.001**	9898	0.94674	0.385	9951
M | 1	5.0881	**< 0.001**	9935	3.3581	**0.002**	9734	1.2555	0.193	9939
P[3] | 1/4	0.32669	0.947	9945	1.6678	0.072	9931	1.5494	0.084	9956
P | 1/2	1.5344	0.121	9820	2.7277	**0.007**	9910	1.1887	0.234	9950
P | 3/4	1.8419	0.050	511	3.0068	**0.003**	5675	1.1224	0.267	9647
P | 1	2.0826	**0.016**	128	3.2549	**< 0.001**	2016	1.0613	0.296	128
D[4] | 1/4	1.7236	0.052	9943	0.62991	0.694	9935	2.1125	**0.013**	9935
D | 1/2	4.6088	**< 0.001**	9775	3.0989	**0.002**	8504	1.5636	0.106	9942
D | 3/4	4.5112	**< 0.001**	6496	1.8603	**0.036**	48	2.4314	**0.013**	9058
D | 1	2.8155	**0.005**	512	1.1825	0.163	16	1.7942	**0.043**	1023
C[5] | 1/4	2.2125	**0.018**	9950	2.3224	**0.010**	9922	0.74486	0.488	9940
C | 1/2	1.8099	0.067	512	1.0684	0.250	2022	0.85692	0.416	64
C | 3/4	0.47734	0.671	16	1.3507	0.496	3	1.4645	0.237	3
C | 1	1.5202	0.223	3	*[11]			1.4859	0.235	3
PC[6] | 1/4	1.5665	0.105	9955	1.232	0.219	9930	0.68796	0.608	9912
PC | 1/2	0.85897	0.440	128	0.7224	0.647	32	0.62043	0.591	64
PC | 3/4	1.4788	0.211	3	1.1595	0.437	2	0.4604	1	8
PC | 1	1.0708	0.468	2	*			0.92404	1	2

[1]Hindgut, [2]mandibular gland, [3]poison gland, [4]Dufour's gland, [5]DCM control, [6]pencil control, [7]comparison of the numbers of ants that followed the first quarter of the gland trail lenght, [8]comparison of the numbers of ants that followed the first half of the gland trail lenght, [9]comparison of the numbers of ants that followed three quarters of the gland trail length, [10]comparison of the numbers of ants that followed the entire gland trail, [11] denominator is 0

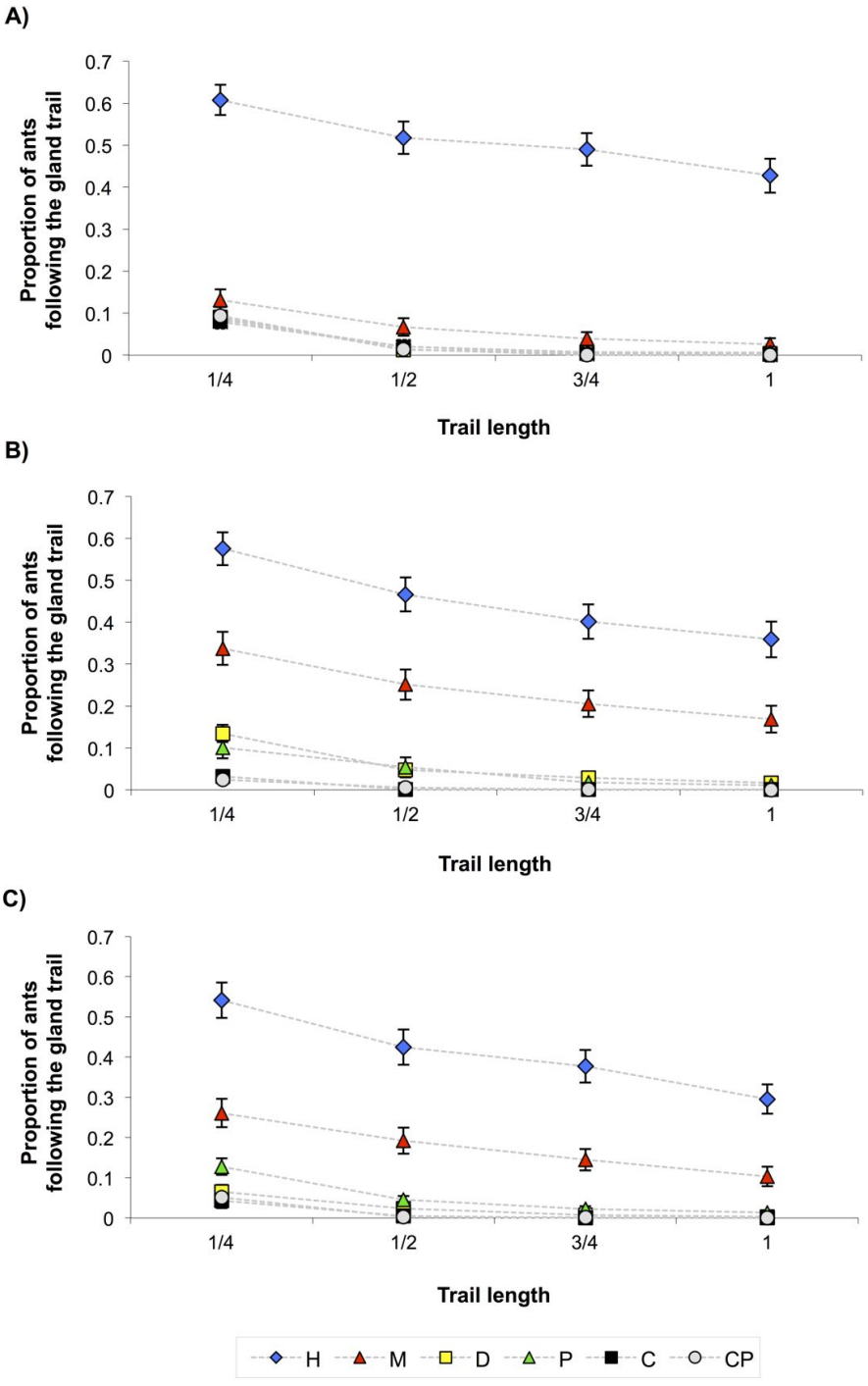


Figure 3.13: Accuracy-in-trail-following experiment with A) the invasive *L. neglectus* and B) the native *L. niger* and C) the native *L. platythorax*. Displayed are the medians and the standard errors of the proportion of ants that followed 1/4, 1/2, 3/4 or the entire length of the S-shaped artificial gland trails. H: hindgut; M: mandibular gland; P: poison gland; D: Dufour's gland, C: DCM control; CP: pencil control.

3.3.3 Alternative-trail-branch experiment

Only a small proportion of ants left the natural trail and turned off to the artificial gland trail. Most ants stayed on the natural trail (Table 3.24). All three ant species were most attracted by hindgut trails compared to the other gland trails (Tables 3.25 and 3.26, Figure 3.14; for the main tests see Tables A.17 and A.18 in Appendix A.1.7.3). Furthermore, the invasive *L. neglectus* and the native *L. niger* were more attracted to mandibular gland trails than by DCM control trails (see effects of (*M, C*) in Table 3.25). While the invasive *L. neglectus* also differed in ant numbers that followed poison gland trails and Dufour's gland trails from the ant numbers that followed DCM control trails there were no differences in ant numbers in the native species (see effects of (*P, C*) and (*D, C*) in Table 3.25). In the native *L. platythorax* even mandibular gland did not attract more ants than the DCM control trails (see effect of (*M, C*) in Table 3.25). Generally, the invasive *L. neglectus* and the native *L. niger* did not differ in their decisions to leave the established natural trail and to follow a branching artificial trail (Table 3.26, Figure 3.14). Compared to the other two species *L. platythorax* was more attracted by poison gland and control trails (see effects of *P* and *C* for (*L. neglectus, L. platythorax*) and (*L. platythorax, L. niger*) in Table 3.26, Figure 3.14).

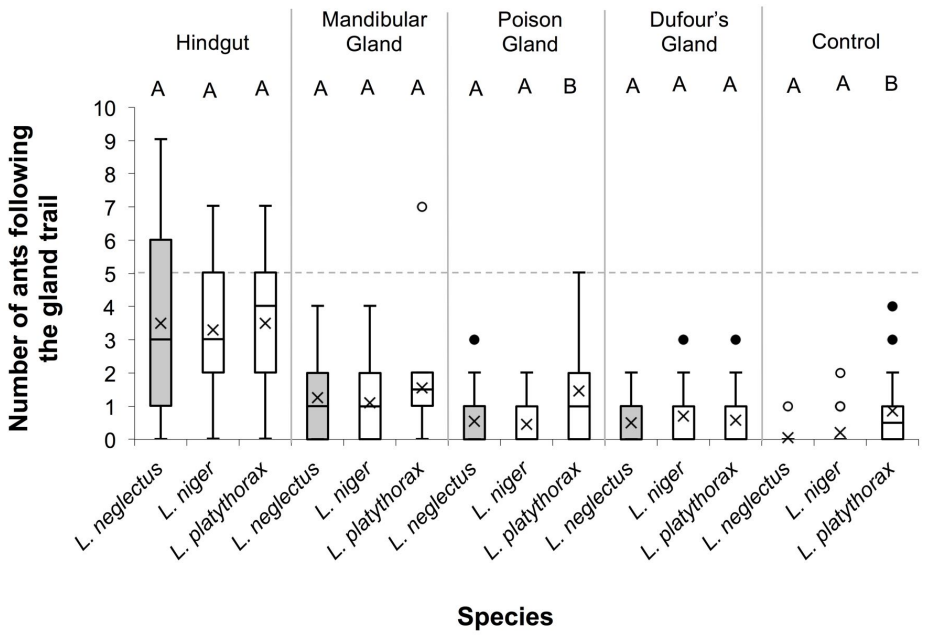


Figure 3.14: Alternative-trail-branch experiment with the invasive *L. neglectus* (boxes shaded in grey) and the native, *L. niger* and *L. platythorax*. Displayed is the number of ants that followed the branch with the artificial gland trail. Box-and-whisker plots with first and third quartiles, median and whiskers (minimum and maximum value); mean (×); values that are more than 1.5 times the interquartile range (IQR) are displayed as outliers (•), values that are more than 3 IQR are displayed as extreme points (◦). Different upper case letters denote significant differences between the species within a gland type (PERMANOVA post-hoc pairwise comparisons, $P < 0.05$).

Table 3.24: Alternative-trail-branch experiment with *L. neglectus*, *L. niger* and *L. platythorax*: Wilcoxon signed-rank test comparing the numbers of ants that followed the natural gland trail with the numbers of ants that turned off a natural gland trail onto a artificial gland trail that consisted of hindgut, mandibular gland, poison gland or Dufour's gland solutions or DCM. The table shows the sample sizes (N), the test statistics (W) and the two-sided Monte-Carlo P-values. P-values less than 0.05 are marked in bold.

Testspecies	**Gland branch**	N	W	**two-sided** P
L. neglectus	Hindgut	20	150.5	**0.024**
	Mandibular gland	20	210.0	**< 0.001**
	Poison gland	20	210.0	**< 0.001**
	Dufour's gland	20	210.0	**< 0.001**
	DCM control	20	210.0	**< 0.001**
L. niger	Hindgut	20	139.0	**0.002**
	Mandibular gland	20	210.0	**< 0.001**
	Poison gland	20	210.0	**< 0.001**
	Dufour's gland	20	210.0	**< 0.001**
	DCM control	20	210.0	**< 0.001**
L. platythorax	Hindgut	20	120.5	**0.003**
	Mandibular gland	20	209.0	**< 0.001**
	Poison gland	20	190.0	**< 0.001**
	Dufour's gland	21	231.0	**< 0.001**
	DCM control	20	210.0	**< 0.001**

Table 3.25: Alternative-trail-branch experiment with the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*. PERMANOVA post-hoc pairwise comparisons of the glands for each species. Compared are the numbers of ants that turned off a natural gland trail to the artificial gland trail. The table shows the test statistics (t), P-values determined by permutations, and the numbers of unique permutations. P-values less than 0.05 are marked in bold.

Comparison	***L. neglectus***			***L. niger***			***L. platythorax***		
	t	P **(perm)**	**Unique perms**	**t**	P **(perm)**	**Unique perms**	**t**	P **(perm)**	**Unique perms**
H[1], M[2]	33.566	**0.002**	28	42.886	**< 0.001**	24	34.708	**0.002**	24
H, P[3]	46.049	**< 0.001**	29	5.884	**< 0.001**	24	37.112	**0.002**	25
H, D[4]	47.749	**< 0.001**	28	51.621	**< 0.001**	24	61.653	**< 0.001**	26
H, C[5]	56.447	**< 0.001**	29	65.392	**< 0.001**	25	51.262	**< 0.001**	24
M, P	2.041	0.069	14	23.631	**0.033**	11	0.2154	0.919	17
M, D	23.486	**0.037**	13	12.996	0.268	12	26.116	**0.005**	26
M, C	42.219	**< 0.001**	13	35.088	**0.001**	11	16.609	0.130	15
P, D	0.19916	1	10	0.97177	0.437	9	24.331	**0.029**	15
P, C	24.443	**0.029**	7	12.955	0.307	7	14.678	0.187	15
D, C	27.809	**0.015**	6	2.107	0.064	9	0.90693	0.438	21

[1]Hindgut, [2]mandibular gland, [3]poison gland, [4]Dufour's gland, [5]DCM control

Table 3.26: Alternative-trail-branch experiment with the invasive *L. neglectus* (boxes shaded in grey) and the native, *L. niger* and *L. platythorax*. PERMANOVA post-hoc pairwise comparisons of the species for each gland type. Analyzed is the number of ants that turned off a natural gland trail to the artificial gland trail. The table shows the test statistics (t), *P*-values determined by permutations, and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

	L. neglectus, L. niger			***L. neglectus, L. platythorax***			***L. niger, L. platythorax***		
Gland	**t**	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**
H[1]	0.26214	0.847	28	$1.70 \cdot 10^{-4}$	1	26	0.31095	0.813	25
M[2]	0.41539	0.783	12	0.68588	0.598	15	11.074	0.349	13
P[3]	0.39874	0.847	10	23.897	**0.028**	15	28.166	**0.009**	15
D[4]	0.77664	0.574	10	0.3034	0.845	17	0.47441	0.720	19
C[5]	11.791	0.490	3	30.882	**0.002**	10	23.231	**0.036**	11

[1]Hindgut, [2]mandibular gland, [3]poison gland, [4]Dufour's gland, [5]DCM control

3.3.4 Single-point-source experiment

Lasius neglectus, *L. niger* and *L. platythorax* showed six different behavioral reactions when passing single squashed glands offered next to a natural trail: *antennating up*, *antennating down*, *contact*, *running back*, *hiding* and *spinning* (Table 3.27). All treatments induced measurable reactions of the ants, although the reactions to the control were rather small (Table 3.28). Most interestingly, the behaviors shown by the invasive *L. neglectus* did not differ from those of the native species, *L. niger* and *L. platythorax*, while the native species differed in their behaviors (Table 3.30; for the main test see Table A.20 in Appendix A.1.7.4). The most diverse behavioral reactions to the different glands were shown by the invasive *L. neglectus* (Table 3.29; for the main test see Table A.20 in Appendix A.1.7.4). In contrast, the native *L. platythorax* behaved rather uniformly to different gland types (Table 3.29).

Being in physical contact with the filter paper was within the three main effects for all treatments (Tables 3.28). Actually, in *L. neglectus* and *L. niger contact* was the main effect of all treatments except of mandibular gland treatments. Physical contact was always associated with searching (*antennating up*) or examination (*antennating down*) behaviors. Especially hindguts induced these behaviors to a large extent in all three species (Table 3.28). The other pheromone sources often induced avoiding behavior like *hiding* or agitation like *spinning*. In *L. platythorax hiding* was the main or second main effect of all treatments except of hindgut treatments. *Lasius neglectus* hid when passing mandibular glands. In *L. niger hiding* was not within the main effects induced by pheromone source treatments. The spinning behavior, although only rarely shown in this experiment, seems to be a form of panic alarm where the ants dash around in erratic patterns (Hölldobler and Wilson, 1990). The spinning behavior was triggered by Dufour's glands in *L. neglectus* and *L. niger* and by poison glands in *L. niger*. A detailed figure with the proportions of all behaviors is given in the Appendix (Figures A.1 and A.2 in Appendix A.1.7.4).

Table 3.27: List of behaviors shown by *Lasius neglectus*, *Lasius niger* and *Lasius platythorax* in the single-point-source experiment

Behavior	**Definition**
antennating up (ant. up)	Ant stops and lifts its antennae
antennating down (ant. down)	Ant examines the filter paper with its antennae
contact	Ant stays more than 2 seconds in contact with the filter paper
running back	Ant recoils and goes back the direction it came from
hiding	Ant runs to the upper side of the wooden bridge
spinning	Ant quickly runs in a semicircle next to the filter paper

Table 3.28: Single-point-source experiment with the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*. Analyzed are the numbers of the behaviors the ants showed when passing the different pheromone sources. The table shows the three main effects of each pheromone source on each species. Mean proportions (Nr. of behavior/ Nr. of ants passing the pheromone source) and standard errors of the means are given in brackets.

Pheromone source	***L. neglectus***	***L. niger***	***L. platythorax***
Hindgut	contact (0.40 ± 0.05)	contact (0.51 ± 0.06)	ant. up (0.14 ± 0.03)
	ant. down (0.16 ± 0.02)	ant. down (0.38 ± 0.04)	contact (0.13 ± 0.04)
	ant. up (0.12 ± 0.02)	ant. up (0.12 ± 0.02)	ant. down (0.08 ± 0.02)
Mandibular gland	ant. up (0.24 ± 0.05)	ant. up (0.26 ± 0.04)	hide (0.10 ± 0.02)
	contact (0.11 ± 0.02)	contact (0.18 ± 0.03)	ant. up (0.09 ± 0.02)
	hide (0.10 ± 0.04)	ant. down (0.13 ± 0.02)	contact (0.03 ± 0.01)
Poison gland	contact (0.16 ± 0.03)	contact (0.56 ± 0.08)	hide (0.08 ± 0.02)
	ant. down (0.13 ± 0.03)	ant. down (0.49 ± 0.07)	ant. up (0.06 ± 0.01)
	ant. up (0.10 ± 0.03)	spin (0.10 ± 0.02)	contact (0.03 ± 0.01)
Dufour's gland	contact (0.40 ± 0.08)	contact (0.78 ± 0.05)	ant. up (0.13 ± 0.03)
	ant. down (0.20 ± 0.06)	ant. down (0.40 ± 0.04)	hide (0.10 ± 0.02)
	spin (0.09 ± 0.04)	spin (0.15 ± 0.02)	contact (0.04 ± 0.01)
Control	contact (0.03 ± 0.01)	contact (0.13 ± 0.03)	hide (0.04 ± 0.01)
	ant. down (0.03 ± 0.01)	ant. down (0.09 ± 0.02)	ant. up (0.03 ± 0.01)
	ant. up (0.03 ± 0.01)	hide (0.04 ± 0.02)	contact (0.02 ± 0.01)

Table 3.29: Single-point-source experiment with the invasive *L. neglectus* and the native species, *L. niger* and *L. platythorax*. PERMANOVA post-hoc pairwise comparisons of the glands for each species. Analyzed are the numbers of different behaviors the ants showed when passing the different gland sources. The table shows the test statistics (t), *P*-values determined by permutations, and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

	L. neglectus			***L. niger***			***L. platythorax***		
Comparison	**t**	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**	**t**	***P* (perm)**	**Unique perms**
H[1], M[2]	3.5153	**< 0.001**	9950	4,3973	**< 0.001**	9939	1.958	**0.025**	9941
H, P[3]	2.8998	**0.002**	9945	1.1353	0.263	9932	2.1355	**0.016**	9955
H, D[4]	0.71606	0.606	9965	3.0368	**0.001**	9945	1.6209	0.079	9933
H, C[5]	6.2443	**< 0.001**	9924	5.5333	**< 0.001**	9938	2.6415	**0.002**	9952
M, P	1.7771	**0.026**	9928	4.499	**< 0.001**	9936	0.80636	0.564	9954
M, D	2.6426	**< 0.001**	9939	7.7976	**< 0.001**	9956	0.75219	0.572	9945
M, C	3.2413	**< 0.001**	9941	3.4427	**< 0.001**	9953	2.0978	**0.010**	9950
P, D	2.0015	**0.021**	9930	1.8721	0.052	9941	1.4338	0.118	9947
P, C	2.9201	**< 0.001**	9938	5.1365	**< 0.001**	9918	1.5229	0.083	9939
D, C	3.5806	**< 0.001**	9925	9.9808	**< 0.001**	9921	2.545	**0.003**	9956

[1]Hindgut, [2]mandibular gland, [3]poison gland, [4]Dufour's gland, [5]filter paper control

Table 3.30: Single-point-source experiment with the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*. PERMANOVA post-hoc pairwise comparisons of the species. Analyzed are the numbers of different behaviors the ants showed when passing the different gland sources. The table shows the test statistics (t), *P*-values determined by permutations, and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

Comparison	**t**	***P* (perm)**	**Unique perms**
L. neglectus, L. niger	1.5809	0.129	9237
L. neglectus, L. platythorax	1.9813	0.074	9531
L. niger, L. platythorax	2.9269	**0.012**	9801

3.4 Analyses of specific gland ingredients

The gland ingredients of three different gland types, i.e. hindgut, poison gland and Dufour's gland, of the invasive *L. neglectus* and the native species, *L. niger* and *L. platythorax*, were analyzed with a coupled gas-chromatograph and mass-spectrometer. Presence of the ingredients were compared among species and among extracts and controls.

Gland extracts and control solutions highly differed for all species and almost all glands (see effects of *C1*, *C2*, *C4*, *C5*, *C6*, *C7* and *C8* in Table 3.31). An exception was the hindgut extract, which did not differ from its respective control solution in either native species, *L. niger* and *L. platythorax* (see effects of *C3* and *C9* in Table 3.31). The comparison of the species has shown significant differences among Dufour's glands and also among hinguts (see effects of *C10*, *C11*, *C12*, *C16*, *C17* and *C18* in Table 3.31). Only the poison gland extracts did not differ among the species (see effects of *C13*, *C14* and *C15* in Table 3.31).

3.4.1 Chemical structure of gland ingredients in *Lasius*

In total, 60 gland ingredients were detected in hindgut, poison glands and Dufour's glands of *L. neglectus*, *L. niger* and *L. platythorax* (Table 3.32): Nine of them were extracted only from *L. neglectus* glands, 26 only from *L. niger* glands and four only from *L. platythorax* glands. *Lasius neglectus* shared five substances with *L. niger* and three with *L. platythorax*. The native species shared five substances. Further eight substances were extracted from all three species. Most substances were extracted from poison glands (31) and Dufour's glands (39). The fewest ingredients were extracted from hindguts (9) with only one hindgut specific component (RI[1] = 1828) in *L.niger*.

Aliphatic hydrocarbons of different lengths (undecane, dodecane, tridecane, pentadecane, heptadecane and hexadecane) and with methyl substituents on different positions (3-methyl-undecane, 5-methyl-undecane, 3-methyltridecane) were identified. While *L. niger* had all of them only few were found in *L. neglectus* and *L. platyhtorax*. Six out of nine hydrocarbons were exclusively found in Dufour's glands. Seven alkene (undecene, tridecene (A), tridecene (B), heptadecene, nonadecene (A), nonadecene (B), squalene) were found in Dufour's glands (6) and in poison glands (2). Tridecene in both configurations were only found in *L. platythorax*. One aldehyde (hexadecanal) and four ketones (3-tetradecanone, 2-tridecanone, 2-heptadecanone and 2-pentadecanone) were extracted from poison glands and Dufour's glands of *L. neglectus* and *L. platythorax*. With the exception of 2-pentadecanone the four chemical compounds were in this context specific to the invasive *L. neglectus*. One alcohol was extracted from poison and Dufour's glands of *L. niger* (4-methyl-dodecanol), one was extracted from Dufour's glands of *L. niger* (undecanol) and of *L. platythorax* (tridecanol) and two were extracted from poison glands of all three *Lasius* species (hexadecanol, hexadecenol). Furthermore, three acetate (farnesyl acetate, hexadecyl acetate, octadecyl acetate),

[1] Retention indices (RI) are used to convert retention times (RT) into system-independent constants

Table 3.31: Comparison of pheromone blends extracted from three pheromone glands, i.e. hindgut, poison gland and Dufour's gland, among *L. neglectus*, *L. niger* and *L. platythorax*. The PERMANOVA design took one factor (*comparison*) into account. Comparisons of interest (between gland extract and control solution; among gland extracts of different species) were pre-defined as contrasts. The table shows the degrees of freedom (df), sums of squares (SS), mean squares (MS), Pseudo-F values, P-values determined by permutations, and the numbers of unique permutations. P-values less than 0.05 are marked in bold.

Source			**df**	**SS**	**MS**	**Pseudo-F**	**P (perm)**	**Unique perms**
Comparison			17	72489	4264	51,289	**< 0.001**	9899
C1:	LN[1]	C(D)[3] vs. D[2]	1	27228	27228	193.27	**< 0.001**	985
C2:	LN	C(P) vs. P[2]	1	0.36158	0.36158	46,472	**0.007**	126
C3:	LN	C(H) vs. H[2]	1	27.2	27.2	11,999	0.346	462
C4:	LNE[1]	C(D) vs. D	1	9765.9	9765.9	30,495	**< 0.001**	255
C5:	LNE	C(P) vs. P	1	10,995	10,995	72,936	**0.009**	126
C6:	LNE	C(H) vs. H	1	7.30	7.30	3.04	**0.030**	1710
C7:	LP[1]	C(D) vs. D	1	10770	10770	146.17	**< 0.001**	1912
C8:	LP	C(P) vs. P	1	0.26263	0.26263	65,024	**0.006**	126
C9:	LP	C(H) vs. H	1	2.16	2.16	0.6759	0.696	462
C10:	LN vs. LNE	D	1	10173	10173	19,921	**< 0.001**	8167
C11:	LN vs. LP	D	1	11690	11690	50,495	**< 0.001**	9421
C12:	LNE vs. LP	D	1	2427.1	2427.1	59,526	**0.004**	8954
C13:	LN vs. LNE	P	1	0.99592	0.99592	4,495	0.053	126
C14:	LN vs. LP	P	1	0.2129	0.2129	19,111	0.146	126
C15:	LNE vs. LP	P	1	0.4811	0.4811	25,218	0.121	126
C16:	LN vs. LNE	H	1	67.6	67.6	58,871	**0.002**	1709
C17:	LN vs. LP	H	1	43.9	43.9	40,537	**0.001**	462
C18:	LNE vs. LP	H	1	20.0	20.0	59,231	**0.007**	1250
Residuals			109	9061.9	83,137			
Total			126	81551				

[1]Species names abbreviations: LNE: *L. neglectus*, LN: *L. niger*, LP: *L. platythorax*; [2]pheromone gland abbreviations: D: Dufour's gland, P: poison gland, H: hindgut; [3]control treatment for the respective pheromone gland;

five esters (acetacidester, acetaundecester, dodecanoacidodecester, hexadecanoicacidester) and one acid (hexadecanoicacid) were identified.

Table 3.32: List of chemical substances extracted from hindgut, poison glands and Dufour's glands of *L. neglectus* (LNE), *L.niger* (LN) and *L.platythorax* (LP).

Substance	Hindgut			Poison gland			Dufour's gland			
	LNE	LN	LP	LNE	LN	LP	LNE	LN	LP	
hexadecanal				+						
nonadecene(B)							+			9 substances specific to *L. neglectus*
RI[1]=1584							+			
RI=1931				+						
RI=2372				+						
RI=2519				+						
RI=2981				+						
3-tetradecanone							+			
2-tridecanone	+			+			+			
acetacidecester								+		
acetacundec-ester								+		
C11H22								+		
dodecanoacido-decester								+		
farnesacetate								+		
heptadecane		+			+			+		
hexadecane								+		
hexadecanoic-acidester					+					
4-methyl-dodecanol					+			+		
3-methyl-tridecan								+		26 substances specific to *L. niger*
5-methyl-undecane								+		
octadecylacetate					+			+		
RI=1459.4					+			+		
RI=1462								+		
RI=1526.7								+		
RI=1594.8								+		
RI=1828		+								
RI=2198.1								+		
RI=2341.5					+					
RI=2384								+		
RI=2423.1								+		
RI=2426								+		
RI=2468					+					
RI=2695.3								+		
undecanol								+		
undecene								+		
2-heptadecanone			+			+			+	
tridecanol									+	4 substances specific to *L. platythorax*
tridecene(A)									+	
tridecene(B)									+	
heptadecene							+	+		
3-methyl-undecane							+	+		5 substances shared by *L. neglectus* and *L. niger*
RI=1929				+	+					
RI=2296				+	+					
RI=2572				+	+					
nonadecene(A)	+		+	+		+	+		+	
2-pentadecanone			+	+		+	+		+	3 substances shared by *L. neglectus* and *L. platythorax*
RI=2123				+		+				

Continued on next page

Table 3.32 Continued from previous page

Substance	Hindgut			Poison gland			Dufour's gland			
	LNE	LN	LP	LNE	LN	LP	LNE	LN	LP	
acetacidodecester		+			+			+	+	
dodecane								+	+	5 substances shared by *L. niger* and *L. platythorax*
pentadecane								+	+	
RI=2167					+	+				
squalene					+	+				
hexadecacetate				+	+	+				
hexadecanoicacid				+	+	+				
hexadecanol				+	+	+				8 substances shared by *L. neglectus*, *L. niger* and *L. platythorax*
hexadecenol				+	+	+				
RI=2482				+	+	+				
RI=2642	+	+	+	+	+	+				
tridecane						+	+	+	+	
undecane	+			+	+	+	+	+	+	

[1] Retention index is shown when the substance is not identified yet

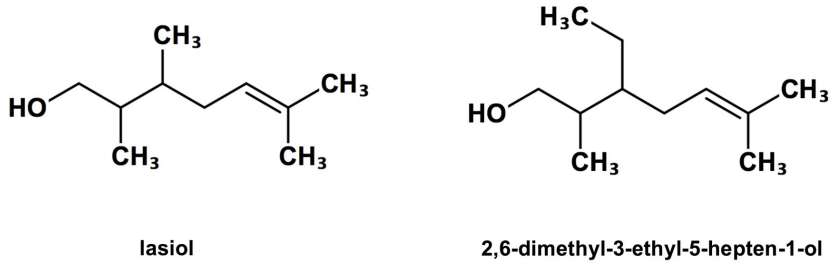


Figure 3.15: Molecular structure of the specific substance 2,6-dimethyl-3-ethyl-5-hepten-1-ol (right) extracted from hindguts of *L. neglectus*. 2,6-dimethyl-3-ethyl-5-hepten-1-ol is a derivate of lasiol (left).

3.4.2 Isolation of species-specific hindgut substances in *L. neglectus*

One-minute retention time[2] (RT) intervals of the *L. neglectus* hindgut chromatogram were tested in bioassays. *Lasius neglectus* ants responded positively on three RT intervals, indicating bioactive substances between minutes 07:00 and 10:00 (Table 3.33). Although bioactivity was not statistically confirmed due to small sample size, 100 % of the ants followed the conspecific trail in minute 07:00 - 08:00 and minute 08:00 - 09:00, and 90 % of the ants followed in minute 09:00 - 10:00.

The three bioactive intervals were subdivided into 30 seconds intervals and tested with *L. neglectus*, *L. niger* and *L. platythorax*. One interval (07:00 - 07:30) was bioactive in *L. neglectus*, but neither in *L. niger* nor in *L. platythorax* (Table 3.33), indicating species-specific bioactive substances for *L. neglectus* in this time span.

With further subdivisions into 15 seconds and 6 seconds intervals the bioactive time span was localized between minutes 07:24 and 07:30 (Table 3.33). Only one substance in the tested interval of the hingut sample was found not belonging to Dufour's gland. By analyzing the mass spectrum of this substance it was possible to identify the chemical structure of a lasiol derivate: 2,6-dimethyl-3-ethyl-5-hepten-1-ol, an unsaturated alcohol with eleven carbons and molecular mass of 170 g/mol (Figure 3.15).

[2] Retention time (RT): the amount of time elapsed from the injection of a sample into the chromatographic system to the recording of the peak maximum of the component in the chromatogram.

Table 3.33: Specifity of hindgut extractions in the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*: Wilcoxon signed-rank test with the number of ant workers that followed either the hindgut trail of *L. neglectus* or the DCM control trail on a Y-bifurcation. The chromatogram of the hindgut extractions were sectioned and re-captured in one minute, 30 seconds and 15 seconds retention time (RT) intervals for testing bioactivity of specific gland compounds. The table shows the test species, the tested sample intervals (RT interval), the numbers of observations (N), the test statistics (W), the *P*-values and the bioactivities.

Species	**RT interval**	**N**	**W**	*P*	**Bioactivity**
	1 minute				
L. neglectus	06:00 - 07:00	3	-	-	no[1]
L. neglectus	07:00 - 08:00	3	-	-	yes[1]
L. neglectus	08:00 - 09:00	3	-	-	yes[1]
L. neglectus	09:00 - 10:00	3	-	-	yes[1]
L. neglectus	10:00 - 11:00	3	-	-	no[1]
L. neglectus	11:00 - 12:00	3	-	-	no[1]
L. neglectus	12:00 - 13:00	3	-	-	no[1]
L. neglectus	13:00 - 14:00	3	-	-	no[1]
L. neglectus	14:00 - 15:00	3	-	-	no[1]
	30 seconds				
L. neglectus	07:00 - 07:30	9	45.0	**< 0.001**	yes
L. neglectus	07:30 - 08:00	9	17.5	0.989	no
L. neglectus	08:00 - 08:30	9	30.5	0.054	no
L. neglectus	08:30 - 09:00	9	6.5	0.937	no
L. neglectus	09:00 - 09:30	6	3.0	0.185	no
L. neglectus	09:30 - 10:00	2	-	-	no[1]
	30 seconds				
L. niger	07:00 - 07:30	9	3.0	0.376	no
L. platythorax	07:00 - 07:30	6	4.0	0.497	no
L. niger	07:30 - 08:00	9	5.0	0.321	no
L. platythorax	07:30 - 08:00	9	9.0	0.229	no
	15 seconds				
L. neglectus	07:00 - 07:15	6	0	0.243	no
L. neglectus	07:15 - 07:30	9	45	**< 0.001**	yes
L. neglectus	07:30 - 07:45	3	-	-	no[1]
	10 seconds				
L. neglectus	07:15 - 07:25	9	45	**< 0.001**	yes
L. neglectus	07:24 - 07:35	7	21.0	**0.031**	yes

[1] Bioactivity was estimated visually

CHAPTER 4

DISCUSSION

Invasion success in ant species is promoted by traits such as high intraspecific aggression, supercolonial structures and high numbers of fertile queens, among others (Section 1.4). Native pest ant species were shown to share these traits with invasive ant species. In urban environments, for example, the odorous house ant *Tapinoma sessile* often exhibits extreme polygyny, forms large supercolonies, and becomes a dominant pest (Buczkowski, 2010). In contrast, in natural habitats *T. sessile* colonies are rather small, monogynous and monodomous and they also coexist with a variety of other ant species (Buczkowski, 2010). Thus, it seems that habitat degradation and urbanization can lead to the development of invasive traits in native ant species (Buczkowski, 2010). For several years, the native ant species *F. fuscocinerea* has been treated as a pest in urban areas of Munich. The results of this dissertation suggest that the striking mass occurrences of this species can similarly be attributed to traits known from invasive ant species. Pronounced interspecific aggression enables *F. fuscocinerea* to dominate other ant species. Furthermore, intraspecific aggression is lacking between individuals originating from sites that were up to 58 kilometers apart, indicating weak or nonexistent colony boundaries.

Although the entirety of invasive traits in ants includes genetic, chemical, morphological and behavioral traits, the role of chemical communication has previously barely been taken into account. Efficient communication is a crucial factor for organizing the complex social behavior in ant colonies and might particularly be pronounced in invasive ant species. Falsifying the hypothesis, the results of this dissertation suggest that the invasive garden ant *L. neglectus* did not show a considerably more sophisticated pheromone communication compared with its native relatives, *L. niger* and *L. platythorax*. Nevertheless, the trail communication of the invasive garden ant seems to be adapted to the exploitation of stable and productive food sources. *Lasius neglectus* showed a higher precision in following hindgut trails than the native species.

Knowledge about the communication system enables a targeted manipulation of pest species in pest control (Nordlund et al., 1981; Smart et al., 2014). By imitating the presence of a signaler, semiochemicals are used to attract individuals of a certain species to trap them, or to discourage them from entering a certain area (Smart et al., 2014). Ideally the control treatment is species-specific in the sense that it exclusively affects the target species. The comparison of the pheromone blends of the invasive garden ant *L. neglectus* and its native relatives, *L. niger* and *L. platythorax*, showed notable differences. The chemical attractant 2,6-dimethyl-3-ethyl-5-hepten-1-ol could unambiguously be assigned to the hindgut of the invasive garden ant *L. neglectus*.

4.1 Competitive advantages of the native pest ant *F. fuscocinerea*

This section is based on Pohl et al. (2018)

Formica fuscocinerea is a European ant species that has recently attracted much attention because of mass occurrences in Southern Germany. Two hypotheses are considered which can explain sudden mass occurences of *F. fuscocinerea* within the native range: A lack of natural competitors enables an unhindered spread of *F. fuscocinerea* in human disturbed habitats. The presence of natural competitors would consequently lead to less dominant *F. fuscocinerea* occurrences, e.g. due to niche differentiation. An alternative hypothesis is that *F. fuscocinerea* possesses behavioral traits commonly attributed to invasive ants, specifically a high interspecific competitive dominance and a supercolonial population structure. This would enable *F. fuscocinerea* to dominate habitats with natural competitors.

Inter- and intraspecific competition in ant communities is mainly over territories and food resources (Hölldobler and Wilson, 1990). Three different competition levels can be distinguished (Vepsäläinen and Pisarski, 1982): Ant species that defend only their nesting space are assigned to the lowest level. Ant species that additionally defend food resources rank on an intermediate level and species that defend entire foraging territories rank on top. High-level competitors are assumed to be in direct competition with other high- and intermediate-level species (Vepsäläinen and Pisarski, 1982). Many *Formica* species are high-level competitors (Seifert, 2007) and this study provides evidence for *F. fuscocinerea* exhibiting this competition level. *Formica fuscocinerea* is territorial and within its territories dominant over other ant species (Section 3.1): In the core area of the *F. fuscocinerea* distribution no other ant species were observed or counted at the baits during the field survey. In the periphery of the core area *Formica fuscocinerea* and *M. ruginodis* occurrences overlapped at baits, however, only spatially but not temporarily.

A large proportion of the species composition in the habitat was explained by the recorded habitat parameters, i.e. temperature, humidity, sun exposure and habitat section which were defined by vegetation and substrate. The distribution of the species could, thus, also be a result of niche differentiation: *Myrmica ruginodis* is a submissive species and can be expected to co-exist with strong competitors due to niche differentiation such as behavioral adaptations (Savolainen et al., 1989; Vepsäläinen and Savolainen, 1990). In an ant community assemblage consisting of *Formica* (*Formica* s. str.) *truncorum*, *Formica* (*Coptoformica*) *exsecta* among others and *M. ruginodis*, the latter was found to shift its foraging activities to lower temperatures and night-times, most likely to mitigate competition against the day-active *F. truncorum* and *F. exsecta* (Vepsäläinen and Savolainen, 1990). In this study, *M. ruginodis* and *F. fuscocinerea* had similar temperature preferences. Since *F. fuscocinerea* was active both day and night, however, *M. ruginodis* would not have benefited from shifting activity times. Niche differentiation might also be indicated by differences in food preferences (Carroll et al., 2011), as observed in *F. fuscocinerea* and *M. ruginodis*. The coexistence of both species in parts of the study area would then be possible. However, the preference for honey in *M. ruginodis* might also be a result from food limitation as *F. fuscocinerea* dominated an area of high carbohydrate availability and monopolized conifer trees with trophobionts. *Myrmica ruginodis* and *L. niger* are known to exploit trophobionts as well (Seifert, 2007). After all, although there were no conspicuous ant trails on other trees in the study area the exploitation of trophobionts by *M. ruginodis* and *L. niger* cannot be excluded.

In contrast to *M. ruginodis*, *L. niger* has been ranked dominantly higher than such species of the subgenus *Serviformica*[1] like *F. fusca*, *Formica lemani* (Savolainen et al., 1989) or *Formica cunicularia*

[1] The subgenus *Serviformica* involves the species *F. lemani*, *F. fusca*, *F. selysi*, *F. cinerea*, *F. fuscocinerea*, *F. cunicularia*, *F. rufibarbis*, *F. picea* and *F. candida*. In contrast to the other *Formica* subgenera, i.e. *Raptiformica*, *Formica* s. str., and *Coptoformica*, species of the subgenus *Serviformica* have an independent colony foundation, and serve as slaves for the slave-making *Raptiformica* and as hosts for the temporary parasitic colony founders, *Formica* s. str. and *Coptoformica*. (Seifert, 2007; Goropashnaya et al., 2012)

(Seifert, 2007). *Lasius niger* is highly adaptable to varying habitat conditions including anthropogenic habitats such that it can regularly be found in urbanized areas (Ambach, 1998; Seifert, 2007; Cerdá et al., 2009; Ślipiński et al., 2012; Vepsäläinen et al., 2008); nest densities of more than 100 colonies per 100 square meter are possible (Seifert, 2007). Thus, *L. niger* can be regarded as a potential competitor of *F. fuscocinerea* in urbanized areas. In the field survey there was no overlap of *L. niger* and *F. fuscocinerea* at the baits, although the area of highest *F. fuscocinerea* occurrence also met the habitat preferences of *L. niger* (namely, incomplete vegetation cover and moderately xerotherm to mesophilic conditions; Seifert, 2007). *Lasius niger* occurred only at one bait at the outer edge of the fourth section. This section was adjacent to a habitat similar to the *F. fuscocinerea* hotspot but not examined in this study. The activity of *L. niger* increased with warmer temperatures. This confirms its preference for open and warm habitats such as the *F. fuscocinerea* core area. *Lasius psammophilus* was found to nest in immediate neighborhood of *Formica cinerea* with distances sometimes shorter than one meter (Markó and Czechowski, 2004). *Formica cinerea* is a related species of *F. fuscocinerea* with a similar nesting pattern, social structure and behavior, and also known for its aggressiveness (Seifert, 2007; Markó and Czechowski, 2004). In this species assemblage, direct interferences and conflicts were avoided due to different biotic (vegetation) and abiotic (temperature and humidity) habitat preferences and also due to different foraging strategies (Markó and Czechowski, 2004). The distribution of *F. fuscocinerea*, *M. ruginodis* and *L. niger* in this study area seems to result rather from territoriality and competitive displacement by *F. fuscocinerea* than from niche differentiation. After all, it should be noted that the field observations are descriptive and further studies of other populations are necessary to allow for more general statements about the underlying causes of species distribution in natural habitats.

The results of the laboratory experiments indicate that interspecific competition plays an important role for species distribution in the studied ant community (Section 3.2.1). *Formica fuscocinerea* was the most dominant species able to monopolize food sources in direct encounters with *M. ruginodis* and *L. niger*. While *M. ruginodis* was able to show some resistance, *L. niger* avoided direct encounters with *F. fuscocinerea* at the food sources. Numerical dominance can strongly affect the outcome of competitive interactions: For example, although *F. cinerea* is a highly aggressive species and physically stronger compared to *L. psammophilus*, the position of both species in a competitive hierarchy is not definite but rather seems to depend on numerical dominance (Markó and Czechowski, 2004). According to its worker numbers *L. psammophilus* behaves either as a subordinate species or as a dominant species, particularly when defending baits against *F. cinerea* (Markó and Czechowski, 2004). Interestingly, *L. psammophilus* is assumed to adopt a lower hierarchical position than *L. niger* (Markó and Czechowski, 2004), since the presence of *L. niger* seems to inhibit aboveground foraging in *L. alienus* sensu Förster (i.e. *L. psammophilus* or one of its sibling species) (Brian et al., 1965). Against expectations, ant numbers of *L. niger* at the food source abruptly decreased as soon as *F. fuscocinerea* showed up as a competitor, although *L. niger* had numerical dominance in the initial phase of the competition. However, colony saturation has to be considered as a possible explanation for decreasing ant numbers during the interference competition part of the EIC experiment. While interference competition seems to play an important role, exploitative competition seems to be less relevant for *F. fuscocinerea*, at least in competitive situations with *M. ruginodis* and *L. niger*. The recruitment ability, which was measured by the number of ants visiting the food source, was not superior in *F. fuscocinerea* compared to *M. ruginodis* and *L. niger*. However, exploitative competition is based on a variety of processes and abilities, e.g. the scouts' discovery capability of new food sources (Holway, 1999; Pearce-Duvet et al., 2011) and the species' load carrying capacity (Nielsen et al., 1982). Thus, further studies are necessary to determine further key attributes of exploitative competition in *F. fuscocinerea*.

So far, the distribution pattern of the three species found at the baits in the study area corresponds to the competition hierarchy resulting from the laboratory experiment: *M. ruginodis*, which is successfully withstanding competition of *F. fuscocinerea* in laboratory experiments to a certain extent, co-occurred on the periphery of the *F. fuscocinerea* range. In contrast, *L. niger* was

strongly affected in foraging by the presence of *F. fuscocinerea* in laboratory experiments. At the same time, there was no overlap in occurrence of *L. niger* and *F. fuscocinerea* in the natural habitat. Although *M. ruginodis* and *L. niger* seem competitively incapable of halting further spread and mass occurrences of *F. fuscocinerea*, other ant species might be able to successfully compete against *F. fuscocinerea*. Competitive abilities and resulting dominance hierarchies of *F. fuscocinerea* and other high-level competitors remain to be examined. In an ant community where *F. cinerea* co-occurred with another strong competitor, *Formica* (*Formica* s. str.) *rufa*, foraging areas were separated by so-called buffer zones, i.e. areas that were avoided by both species, most likely to mitigate competitive interactions (Czechowski and Markó, 2005). Since *F. fuscocinerea* and *F. cinerea* seem to be mutually exclusive in their distribution in Germany, Austria and Switzerland (Seifert, 2007), the latter might be able to restrict further spread of *F. fuscocinerea*.

The high densities of *F. fuscocinerea* ants in infested areas (up to 200 nest entrances/m^2; personal observations) seem to speak against intraspecific competition, particularly in territorial species (Boulay et al., 2010; Heller, 2004; Lopez-Sepulcre and Kokko, 2005). However, large polygynous and polydomous colonies and even supercolonial structures are described in species of the *Formica* subgenera *Coptoformica*, *Formica* s. str. and *Serviformica* (Cherix, 1980; Higashi and Yamauchi, 1979; Lindström et al., 1996; Markó et al., 2012). *Formica fuscocinerea* has already been known to form large polydomous colonies (Seifert, 2007). The absence of aggression among *F. fuscocinerea* ants from the study area near Dachau indicates the presence of a polydomous colony (Section 3.2.3.1). Surprisingly no sign of aggression or territorial displays (Le Moli and Mori, 1986; Le Moli et al., 1982; Le Moli and Parmigiani, 1982) was detected among *F. fuscocinerea* populations separated by as much as 58 km. This points towards a lack of distinct behavioral boundaries among ants of physically separated nests as it is also known from many invasive ant species when they form supercolonies (Holway et al., 2002). *Formica* (*Formica* s. str.) *paralugubris* likewise does not exhibit distinct aggression towards conspecifics (Holzer et al., 2006). It nevertheless distinguishes non-nestmates from nestmates by longer antennation bouts (Holzer et al., 2006). In *F. fuscocinerea* there were neither differences in the number of neutral interactions that included antennation nor in the number of peaceful interactions between nestmates and non-nestmates of distant nests and populations, respectively. However, due to small sample sizes of the nestmate controls, minor differences could not be excluded. Furthermore, fast antennation was not analyzed seperately but assigned to neutral interactions together with slow antennation and mere physical contact. Thus, further aggression tests and particularly genetic analyses are necessary to verify supercolonies in *F. fuscocinerea* populations in Germany. Supercoloniality supports the ecological dominance of the most destructive invasive ant species, such as *A. gracilipes*, *L. humile* or *L. neglectus* (Cremer et al., 2008; Holway et al., 2002), and might also be a key trait in explaining the success of *F. fuscocinerea*. Supercolonial species are not only able to out-compete other species due to numerical superiority, they can also compensate for local losses in ant numbers by an intense exchange between connected nests (Myers et al., 1998; Souza et al., 2008; Vega and Rust, 2003). This implies that local eradication programs likely result in a rapid recovery of populations, as has been observed for *F. fuscocinerea* in Munich, due to re-colonisation of ants from adjacent areas.

The combined findings of the field and laboratory studies thereby imply that even in the presence of competitors *F. fuscocinerea* has the potential to colonize and dominate habitats. Habitats with low biotic resistance (Holway, 1998a; Moller, 1996; Rowles and O'Dowd, 2006) are at a special risk of colonization, since *L. niger*, one of the most common synanthropic ant species, does not seem to compete successfully against *F. fuscocinerea*. The traits of *F. fuscocinerea* reported in this thesis, such as a highly polydomous population structure, the lack of behavioral boundaries among physically separated nests, high foraging efficiency, and interspecific dominance are also traits which are well known of invasive ants (Cremer et al., 2008; Errard et al., 2005; Holway et al., 2002; Passera, 1994; Tsutsui and Suarez, 2003). Since these traits are believed to be key factors for the dominance of invasive ants, there is also the potential of further spread and the formation of mass occurrences, particularly in anthropogenic areas, in *F. fuscocinerea*. In contrast to most invasive ant species,

however, *F. fuscocinerea* is able to resist winter temperatures well below 0 °C. Thus, a particular risk of spread exists for temperate regions in middle and northern Europe.

4.2 Competitive advantages of the invasive pest ant *L. neglectus*

Another pest species attracting particular interest in Europe is the invasive garden ant *L. neglectus*. *Lasius neglectus* is already known to exhibit numerous traits typical for invasive ant species. This thesis investigates and compares the competitive abilities among the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*. Most competitive interactions of ant species take place during foraging. Foraging is a complex yet essential process, opening numerous ways to display competitive advantages such as the ability to rapidly discover new food sources, to effectively recruit nestmates, to collectively exploit food sources, to displace competitors and to successfully defend territories and food sources against intruders.

Interestingly, the invasive *L. neglectus* did not have a superior discovering speed compared to the native species, *L. niger* and *L. platythorax*. In this study *Lasius neglectus* was instead found to require more time to find new food sources than the two native species (Section 3.2.2). This suggests that *L. neglectus* rather benefits from numerical superiority than from discovery speed when competing for food sources. In the invasive crazy ant *A. gracilipes* foragers discovered food sources faster in the range of the supercolony but not at the colony boundaries (Drescher et al., 2011). Similar patterns were found for the invasive ant *S. invicta*, where food discovery times decreased with the number of workers (Morrison, 2000). Not surprisingly, the probability to find a food source depends on the number of foraging workers (Morrison, 2000; Holway and Case, 2001).

A high number of foragers supports fast and efficient exploitation of food sources. A flexible and efficient communication system in this context would help at first to mobilize and then to guide a large quantity of nestmates to valuable food sources, and win competition because of numerical advantage. However, even small ant colonies that yet do not exhibit numerical superiority, e.g. in the initial phase of introduction, should have advantages due to a sophisticated communication system. Actually the communication system is considered to be a key factor making ants one of the most successful organisms on earth (Hölldobler and Wilson, 1990). In ants as well as in other species, communication is crucial in the competition for resources, like mating partners, nesting sites or food sources. Thus, superior communication skills should be beneficial in any case, and might also play a key role in the dominance of invasive species. The invasive bird species the Red-billed Leiothrix, *Leiothrix lutea*, was found to be acoustically dominant in an indigenous bird community (Farina et al., 2013). Vocal communication is costly as more energy must be spent in noisy than in quiet areas (Brumm and Slabbekoorn, 2005; Brumm, 2004). Especially urbanized areas exhibit pronounced levels of ambient noise, which is largely of anthropogenic origin and exerts selective pressure on avian acoustic signals (Patricelli and Blickley, 2006; Ryan and Brenowitz, 1985). Thus, bird species like the house crow *Corvus splendens*, which is invasive in Singapore, must have energetic resources to successfully cope with energy-demanding communication in urban areas. Playback of calls of the invasive American bullfrog *Lithobates catesbeianus* led to changes in the acoustic niche of the native frog community (Medeiros et al., 2016). Acoustic noise of invasive species can increase physiological stress and decrease reproductive success in native species comparable to effects of anthropogenic noise (Medeiros et al., 2016). Furthermore, shifting frequencies can provoke higher energy costs resulting in lower fitness of native species (Bosch and Riva, 2004). Hence, communication is a quite effective tool to dominate communities.

Ant species that exploit a variety of prey and food sources benefit from a quick adjustment of their foraging strategies (Witte et al., 2010). A pronounced opportunistic foraging behavior can be found in many invasive ant species (Holway et al., 2002). Thus, the invasive *L. neglectus* can be expected to communicate in a different way compared to the native, *L. niger* and *L. platythorax*. In order to detect possible differences in the pheromone communication of *L. neglectus*, *L. niger* and

L. platythorax, four different pheromone sources (mandibular gland, poison gland, Dufour's gland and hindgut) were analyzed and compared (Section 3.3).

All experiments confirmed a superior attraction of hindgut trails. The exceeding attraction of hindgut trails was not unexpected as the hindgut plays an important role in the production of trail pheromones in formicine ants (Section 1.5). The invasive *L. neglectus* had a higher accuracy in following S-shaped hindgut trails compared to the native *L. platythorax* which had a less accurate trail following. This poses the question of whether a more or a less accurate trail following ability is more beneficial. A less accurate trail following ability could be advantageous especially when being associated with a higher attraction to new routes. This enables the ants to quicker react to newly discovered food sources. *Lasius neglectus* did not show a strong interest in exploring new routes: Although on average one third of *L. neglectus* ants followed alternative hindgut trails the native ants showed comparable attraction. In general, there was no higher attraction to new routes in the invasive *L. neglectus* than in the native species. In contrast, the native *L. platythorax*, the ant species that showed a less accurate S-shaped trail following also showed a higher attraction to new routes made of poison gland extractions or control solutions when they were offered next to established foraging trail. The invasive garden ant *L. neglectus* is known to extensively exploit trophobionts. The value of trophobiosis is its mutualistic nature, implying the care and protection of hemiptera against predators by the ants in return for carbohydrate-rich food droplets. Thus, once established trophobionts represent a stable, stationary and productive food source. A higher trail accuracy and loyalty should in this case be more advantageous than a faster occupation of new trail routes as it enables the fast and efficient exploitation of the established food source.

The mandibular gland was the second most attractive gland source, yet rather in the native than in the invasive species. The difference was salient when the ants had to accurately follow artificial S-shaped pheromone trails. On average, twice as many ants followed the mandibular gland trail in the native ant species than in the invasive species. The fact that ants followed mandibular gland trails *per se* is notable as this gland is rather well known for containing alarm pheromones, cues related to nestmate recognition or in leaf-cutting ants even antimicrobial substances (Section 1.5). All of these application areas are plausible as mandibular glands are connected to the mandibles on the head: While attacking using the mandibles, ants can easily spray pheromones from their mandibular glands towards the perceived threat. In this way they mark the source of danger either to warn nestmates or to induce attacks from nestmates. Nestmate recognition cues from the mandibular glands can also easily be distributed on the cuticular surface, e.g. during grooming its own or a nestmate's body parts. Last but not least, detrimental microbes in fungus gardens are a serious threat to leaf-cutting ants, as they live in a highly evolved mutualism with the fungus (Rodrigues et al., 2008). Besides other mechanisms, these ants evolved antimicrobial compounds produced in the mandibular glands that can easily be applied during cleaning the fungus (Rodrigues et al., 2008). Ants usually use their abdomen or their legs but not their heads to deposit pheromones on the substrate to establish foraging trails (Hölldobler and Wilson, 1990). However, recruiting nestmates for food exploitation is a process with different steps: When a forager finds a valuable food source it tries to take a sample and then lays a pheromone trail back to the nest and finally it prompts nestmates, e.g. by providing food samples, to follow the pheromone trail (Hölldobler and Wilson, 1990). In this way it is possible that foragers equip food samples with mandibular gland pheromones when feeding nestmates for recruiting purposes. Furthermore, the food source itself also comes into contact with the ants' mandibles. Marking productive food sources with mandibular gland pheromones is, hence, also conceivable. The evolution of an informative value of mandibular gland pheromones in the context of food source exploitation is possible because they are also used for nestmate recognition and, thus, do not exclusively trigger aggressive behavior. Furthermore, alarm pheromones, especially those that trigger aggressive but not primarily escaping behavior, need to contain attractive compounds to a certain extent to recruit supporters in defense situations. Alarm pheromones are usually short molecules with low molecular weight and simple structures, e.g. terpenoids, aliphatic ketones and esters (Verheggen et al., 2010). High volatility helps

to immediately contact nearby nestmates since these molecules tend to spread rapidly in close surroundings. But it also means that their impact is only short-lasting, which is important when transmitting information whose validity is limited in time. When offered in high concentrations in the point-source experiment mandibular gland contents triggered avoiding behavior in the invasive *L. neglectus* and the native *L. platythorax*. In the native *L. niger* attentive behavior was salient.

The other two pheromone sources, the Dufour's gland and the poison gland, induced no accurate trail-following. As in other formicine ants, the Dufour's gland of the studied *Lasius* species contains high concentrations of the alarm pheromone undecane. N-undecane is known to act on short distances and to trigger alarm behavior. Although ant species generally vary in their response towards disturbances (Hölldobler and Wilson, 1990), alarm behaviors can be assigned to one of two behavioral categories: *panic alarm* and *aggressive alarm* (Wilson and Regnier, 1971). While aggressive alarm requires movement towards the source of disturbance and is often associated with attacks, panic alarm is characterized by erratic non-directional movements and excited bursts of running resulting in a rapid scattering of the colony (Vander Meer and Alonso, 1998; Wilson and Regnier, 1971). All three *Lasius* species showed genuine agitation, i.e. spinning and hiding behavior, when they were exposed to high concentrations of Dufour's gland contents. Especially the spinning behavior can be interpreted as panic alarm. In formicines poison glands produce formic acid. Formic acid is actually used for different purposes including defense, but also trail marking and recruitment (Verheggen et al., 2010). In contrast to n-undecane, formic acid acts on long distances. In the invasive *L. neglectus* poison gland extracts were even less attractive to the ants than Dufour's gland extracts, although the effects of the pheromones seems to be concentration dependent with higher attractivity of higher concentrations.

The invasive *L. neglectus* seems to rely primarily on pheromones produced by hindgut during trail communication. However, mixing pheromones of hindgut and other glands or using pheromones of different physical properties could enable the encoding of more complex kinds of information. The invasive African big-headed ant *P. megacephala*, for example, uses two pheromones with different decay rates (Dussutour et al., 2009). The choice between the longer-lasting pheromone that elicits weak recruitment and the short-lasting pheromone that elicits strong recruitment is assumed to confer *P. megacephala* the advantage to quickly react to changing foraging conditions (Dussutour et al., 2009). The same pattern was found in the invasive crazy ant *P. longicornis* which also attracts more attention for its fast recruitment than for its aggressive behavior when competing for food resources (Witte et al., 2007). In contrast to other formicine ants where poison and Dufour's glands produce pheromones that induce alarm behavior, in *P. longicornis* both glands produce attractants with different strength and duration rates (Witte et al., 2007). The duration rate of the pheromones and the composition of pheromones in natural trails are factors of the pheromone communication that were not considered in this study, though.

The analysis of the pheromone glands and their usage does not reveal a more complex trail communication in the invasive *L. neglectus*. Yet, it was expected that competitive dominance due to more efficient communication skills are noticeable during the exploration part of the EIC experiment (Section 3.2.2). In the field, invasive ants were found to recruit more ants to more food sources compared to native ant species (Holway, 1998a; Porter and Savignano, 1990; Holway and Suarez, 1999). This difference could not be confirmed for standardized colony sizes of *L. neglectus*, *L. niger* and *L. platythorax*. The invasive *Lasius* species did not recruit more ants to the food source compared to the two native species. Also, no differences in the change of ant numbers over time was detected among the species, which is an indication of a similar recruitment behavior. Nevertheless, in the invasive *L. neglectus* and in the native *L. niger*, the increase of ants at the food source had not yet reached saturation at the beginning of the interference part of the experiment. Thus, advantages might have only become more apparent over longer time spans.

Interspecific competition abilities become crucial when food sources already occupied by another species are discovered or when other species show up at the own food source. Invasive ants, including *L. neglectus*, are known to displace other ants by means of high aggressive behavior (Cre-

mer et al., 2006; Holway, 1999; Holway et al., 2002; Human and Gordon, 1996; Passera, 1994; Rowles and O'Dowd, 2006; Holway and Suarez, 1999; Hölldobler and Wilson, 1977; Human and Gordon, 1999). However, so far it was not clear whether this pronounced aggression in *L. neglectus* is merely a result of numerical dominance. For this study, standardized sizes of competing colony fragments were used to prevent advantages solely through higher worker numbers.

During the EIC experiments' exploitation part, the explorer species had the opportunity to occupy and exploit the food source undisturbed with full strength. Then at the beginning of the interference competition part the competitor arrived with single scouts at the food source. In each trial the competitor species was outnumbered by the explorer species during the first encounters. Even without an initial numerical advantage *L. neglectus* was able to take over food sources occupied by *L. niger* in the course of the experiment. *Lasius platythorax* could not as easily be displaced from food sources. Thus, *L. platythorax* showed a higher resistance against attempted takeovers by the invasive *L. neglectus* than *L. niger*. The higher competitive ability of *L. platythorax* was also visible in encounters of single ant individuals where *L. platythorax* showed a similar aggressive potential to *L. neglectus*. However, the native species' disposition to act aggressively seems to be situational: As long as the native species occupied the food source, and had to merely defend this food source against small numbers of intruding *L. neglectus* ants, they behaved highly aggressively. In this situation *L. platythorax* and *L. niger* showed similar aggression as *L. neglectus*, even though *L. niger* was eventually outcompeted and had to abandon the food source in the long run.

The competitive abilities of the invasive and the native species differed more clearly when the species showed up as competitors. Both native species acted notably submissively when arriving at food sources that were already occupied by the invasive *L. neglectus*. Thus, the native species seem to modify their investment according to the competitive situation which might be reflected in the current group strength. In contrast, the behavior of the invasive *L. neglectus* was driven by pronounced aggressiveness, though it was outnumbered at least at the beginning of the encounters. *Lasius neglectus* immediately started to fight when intruding food sources that were already occupied by *L. niger* or *L. platythorax*. This is of particular interest since a single ant's motivation to start a fight typically depends on the number of allies relative to the number of competitors (Tanner, 2006; Tanner and Adler, 2009). In fact, the aggressive disposition of *L. neglectus* is not entirely explained by numerical superiority but represents an attribute of a single ant individual. In one-on-one encounters, *L. neglectus* showed the highest levels of aggression. Consequently, even in small groups *L. neglectus* has the potential to aggressively impair established native species, like *L. niger* and *L. platythorax*. However, it is not possible to determine the actual influence of the intruding competitor species on the explorer species in this experiment: Although it seems that the occurrence of the invasive *L. neglectus* led to a reduction of native ant numbers at the food sources, the decrease could also be a result of colony saturation. Nevertheless, different native species affected *L. neglectus* to different extents. The competition of *L. platythorax* led to a stronger decline of *L. neglectus* ant numbers than the competition of *L. niger*. Thus, *L. neglectus*, seemed to be competitively more dominant over *L. niger* than over *L. platythorax*.

The invasive garden ant *L. neglectus* does not seem to escape the trade-off between exploitation and interference competition. Against expectations, *L. neglectus* is not superior in quickly finding new food sources. Its competitive dominance is rather based on pronounced aggressive behavior in direct encounters with competitors. This is in line with the discovery-dominance trade-off found among *L. neglectus* and three other widespread invasive species, i.e. *L. humile*, *W. auropunctata* and *P. megacephala* (Bertelsmeier et al., 2015): *L. neglectus* was the second most dominant species of the four species in direct encounters, but also needed the second most time to discover food sources. This study thereby shows that the aggressiveness of *L. neglectus* is an intrinsic trait of the individual ant. Hence, even small groups of ants are able to face off competitors. Consequently, the larger the colonies of the invasive garden ant the greater will be the negative influence on native ant species as numerical dominance will enhance the competitive strength.

The role that pheromone communication plays in the dominant occurrence of *L. neglectus* could

not be conclusively clarified. It seemed that use and the effects of the pheromone gland substances are similar in the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*. However, even minor differences can have a great impact. A higher accuracy in trial following as shown for the invasive *L. neglectus* could offer the crucial advantage during exploitative competition. Future studies are necessary to focus on further aspects of the pheromone communication such as the constitution and durability of natural pheromone trails.

4.3 An alternative strategy to control the invasive pest ant *L. neglectus*

Biological invasions can cause severe and irreversible loss of biological diversity. Consequences may not be immediately apparent but can be far more wide-reaching than one might assume in the beginning. A striking example is the European honeybee *Apis mellifera*, which has been deliberately introduced around the world for its pollination service. Although it seemed *Apis mellifera* had simply taken the place of native pollinators, special needs of native plants make this bee a bad substitute (Buchmann et al., 2012). Even human food production suffers from the introductions of *Apis mellifera* and the displacement of native species, as a significant proportion of agricultural crops depend on wild insect pollinators (Buchmann et al., 2012). Nowadays, negative impacts of introduced honeybees are well-known (for an overview see Russo, 2016) turning the European honey bee into a subject of increased control efforts (Wenner et al., 2009).

At worst, native ecosystems are already damaged when treatments are applied to control invasive species. In Bavaria, the number of butterfly species has decreased by 13 percent since the middle of the 18th century – most of that loss has happened in the past 25 years (Haslberger and Segerer, 2016; Habel et al., 2016). Thus, a declared intent of control strategies should be to not further impair the sensitive native community by the control treatment itself. Unfortunately, the fight against insect pest species, also the control of invasive ant species, still primarily relies on broad-spectrum insecticides. As a consequence, the native insect community is additionally weakened, especially in cases of large-area applications. To avoid potential negative ecological side effects, the biology of the target species and of the affected ecosystem should be known and taken into account when developing and applying control programs.

Especially in times of increasing awareness for the protection of endangered species, there has been a growing demand for alternative control strategies that minimize collateral damage. Much research has focused on maximizing detrimental effects on target species while simultaneously minimizing the risk on the environment and other species when applying insecticides (e.g. Buczkowski et al., 2014; Knight and Rust, 1990). For example, a novel type of bait station was developed in order to control the invasive Argentine ant *L. humile* on White Beach in California, which is a nesting habitat for the endangered sea bird, the California least tern *Sterna antillarum* (Choe et al., 2010). The idea behind the construction, which contained a slow-acting contact toxin, was to not impede the ants' foraging activity but to provoke the transfer of the toxin to nestmates via grooming and necrophoresis (Choe et al., 2010; Choe and Rust, 2008). The functional duration of the bait stations were extended as they did not fill up with dead ants any longer. Furthermore, other insects than ants were not impaired and a contamination of the environment was prevented. Nevertheless, there was still the risk that ant species other than the target species came into contact with the toxic baits through foraging activities. The use of specific attractants and repellents in the control of insect pest species has been identified as a promising tool for reducing insecticide contaminations in the environment since the early 1960s (Beroza, 1972; Hocking, 1963; Jacobson, 1966). Especially sex attractants turned out to be effective for the control of flying insects like moths or beetles (Beroza, 1972). Their reproductive success highly relies on the ability to locate mates even over long distances such that at least one sex usually exhibits specialized olfactory organs. Since sex attractants are normally highly specific to avoid attraction of wrong recipients they are very suitable for species-specific control purposes (Beroza, 1972).

The results of the EIC experiments conducted as a part of this thesis reveal that the invasive garden ant *L. neglectus* affects the black garden ant *L. niger* more negatively than the sister species *L. platythorax* (Section 3.2.2). Although the invasive *L. neglectus* is still mainly distributed in anthropogenic influenced habitats, like botanical gardens, chances are that the species spreads into natural habitats. Competitive encounters are then more likely to happen with the native *L. niger* than with *L. platythorax*, a species that avoids cultural habitats but can be found in woodlands, bogs and fens. A species-specific control strategy for the invasive *L. neglectus* thus appears advisable.

In some ant species, especially in invasive types, mating takes place within the colony such that there is no need for males to locate females over long distances or even to leave the secure environment of the nest area (Section 1.4). Thus, the usage of sex pheromones to control invasive ants is less promising. However, ants have a pronounced olfactory system as the coordination within a social society to a large part relies on olfactory information (Hölldobler and Wilson, 1990). Attractants play an important role in the ants pheromone trail communication. Since a multiple glandular origin is also assumed for trail pheromones in ants (Section 1.5), the chemical substances of three important gland sources, i.e. poison gland, Dufour's gland and hindgut were analyzed and compared among the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax* (Section 3.4).

Numerous chemicals extracted from poison and Dufour's glands were identified only in one of the three *Lasius* species. That is notable since in formicine[2] ants both glandular sources are associated with the production of alarm pheromones, and alarm pheromones are not considered to be highly species-specific (Vander Meer and Alonso, 1998; Blum, 1969). Especially species that co-occur in the same habitat should have benefits when also responding to allospecific alarm pheromones (Vander Meer and Alonso, 1998; Blum, 1969). Nevertheless, several ant species show higher response to their natural pheromones than to closely related chemical compounds (Blum, 1969). Although the majority of chemical compounds identified in this study were not tested for bioactivity, comparison with literature allows conclusions about their utilization in pheromone communication.

Well-known compounds of the Dufour's glands in several subfamilies of ants are aliphatic hydrocarbons within the range of C_9 to C_{27} (Attygalle and Morgan, 1984). In this study, aliphatic hydrocarbons of different lengths and with methyl substituents on different positions were found in Dufour's glands of all three *Lasius* species. As in other formicine ants undecane was the major component and likely plays the major role in alarm communication (Attygalle and Morgan, 1984). Alkene, those hydrocarbons containing a C$=$C double bond, have in contrast to alkanes a restricted rotatability within the molecules (Schwister et al., 2005). Aside from molecules with different positions of double bonds, cis-trans-isomers can have different chemical properties. Seven alkenes were identified from Dufour's glands and poison glands in this study. Tridecene extracted only from *L. platythorax* Dufour's glands, is also known from *L. flavus* (Bergström and Löfqvist, 1970). Nonadecene, which was found in two configurations in this study, with one configuration found specifically in the invasive *L. neglectus*, has been also found in *Lasius alienus* (Bergström and Löfqvist, 1970). However, the configurations of the molecule in *L. alienus* are not clear. Blum (1969) worked out the importance of aldehydes and ketones in the alarm communication of social insects. One aldehyde and four ketones were identified from poison glands and Dufour's glands in this study. With the exception of 2-pentadecanone, they were specific to the invasive *L. neglectus*. However, at least one of these specific ketones, 2-tridecanone, is also a major component known from other ant species, e.g. *L. alienus*, *F. rufibarbis*, *Gigantiops destructor*, and induces alarm behavior in *Acanthomyops claviger* (Bergström and Löfqvist, 1970; Attygalle and Morgan, 1984; Blum, 1969).

Regular hydrocarbons are largely nonpolar (Schwister et al., 2005). Thus, especially short chains are very volatile and perfectly suited for applications where information needs to spread quickly. On the other hand, they have a weak solubility in water (Schwister et al., 2005). Alcohols have both, a hydrophilic OH-group and a lipophilic alkyl radical. Thus, they can be mixed with water and with

[2] The subfamily Formicinae contains species of the genus *Formica* and *Lasius*, amongst others.

hydrocarbons. Five alcohols were idendified from poison glands and Dufour's glands in this study. Furthermore, three acetates, five esters and one acid were extracted from the ants glands. Farnesyl acetat, has formerly been found in Dufour's glands of *L. niger* (Bergström and Löfqvist, 1970). Due to its special nature, this component was suggested to have a specific biological function (Bergström and Löfqvist, 1970). In contrast to Bergström and Löfqvist (1970), who assumed a common glandular origin of octadecyl acetat and hexadecyl acetat in *L. niger* this study clearly assigned hexadecyl acetat to poison gland while octadecyl acetat was found in both, poison gland and Dufour's gland. Hexadecyl acetat seems to be more widespread among ants than octadecyl acetat: While octadecyl acetat was specifically found in *L. niger*, hexadecyl acetat was found in all three *Lasius* species and was also reported from *L. alienus* (Bergström and Löfqvist, 1970). Both hexadecyl acetat and octadecyl acetat were identified as compounds of sex pheromone blends of the lightbrown apple moth, *Epiphyas postvittana* (El-Sayed et al., 2011). Several fatty acids (hexanoic, heptanoic, octanoic, nonanoic, decanoic, dodecanoic) have been identified as active trail pheromone components in *L.fuliginosus* (Huwyler et al., 1975). No acids have been found in rectal fluids of *L. niger* (Huwyler et al., 1975). In this study, hexadencanoic acid was isolated from poison glands of all three *Lasius* species. Bioactivity of this fatty acid as well as of all other isolated chemical substances has to be evaluated in future studies. Furthermore, the chemical structure of numerous components could not be conclusively clarified.

Unfortunately, there were major issues with the isolation of any chemical substance that can be explicitly and exclusively assigned to hindgut. The issue resulted either from very low substance concentrations in hindguts or from unsuitable extraction methods. The behavioral analyses of the pheromone glands determined the hindgut and rectum as the structures containing substances with the highest attraction in pheromone trail experiments (Section 3.3). Furthermore, hindgut trails of *L. neglectus*, *L. niger* and *L. platythorax* turned out to be species-specific, as the ants showed greater preference for following trails created by their own glands. However, one substance, 2,6-dimethyl-3-ethyl-5-hepten-1-ol was found to be specifically attractive for the invasive ant *L. neglectus*. 2,6-dimethyl-3-ethyl-5-hepten-1-ol is a derivate of lasiol, which is an acyclic monoterpenol that has been found to be a component of mandibular gland secretions of *Lasius meridionalis* (Lloyd et al., 1990). An isomer of lasiol, citronellol, is also a well-known volatile component from several *Lasius* species (Lloyd et al., 1990). The substance 2,6-dimethyl-3-ethyl-5-hepten-1-ol is, thus, a credible candidate for being responsible for the species-specifity of pheromone trails in *L. neglectus*. Its suitability for the use in bait control, however, needs to be tested in future studies.

CHAPTER 5

CONCLUSION AND FUTURE WORK

The objective of this thesis is gaining a better understanding of the mechanisms that enable pest ants and invasive ant species to acquire extraordinary dominance. Profound knowledge about the target species' biology contributes to sustainable control programs. This thesis provides new insights in the competitive abilities of the native pest ant species *F. fuscocinerea* and of the native garden ant *L. neglectus*. The findings raise new scientific issues and prospects for future research some of which are discussed in the following sections.

5.1 Extending the data collection for *F. fuscocinerea*

This thesis investigates mechanisms that enable the native ant species *F. fuscocinerea* to occur as a pest in Southern Germany. Two main research questions are addressed concerning competitive abilities of *F. fuscocinerea*: Does *F. fuscocinerea* primarily spread in areas with low competitive pressure (e.g. playgrounds, parks, etc.) or is it also able to become dominant in more competitive environments? Does *F. fuscocinerea* features special population structures that facilitate fast population regenerations as known from invasive ant species? The results of the field study show that even in the presence of ant competitors *F. fuscocinerea* is able to inhabit and defend a profitable territory. Controlled laboratory experiments confirm the ability of *F. fuscocinerea* to dominate the two native species, *M. ruginodis* and *L. niger*. However, other ant species known to be competitively dominant have not yet been compared with *F. fuscocinerea*. Further studies on the competitive abilities of *F. fuscocinerea* are required to better understand possible effects of *F. fuscocinerea* on other ant communities. Furthermore, the study of *F. fuscocinerea* in other habitats may provide more insights in the relevance of niche differentiation and competitive displacement as influencing factors.

Formica fuscocinerea is found to have colony structures that resemble supercolonies of invasive ant species. While supercoloniality in ants increases competitive dominance it complicates control: Weakened ant nests in treated areas can quickly recover through adopting colony members from neighboring nests in untreated areas. Although this thesis investigates two populations separated by as much as 58 km, colony dimensions of *F. fuscocinerea* still remain unknown. Genetic surveys could help to unravel colony structures of *F. fuscocinerea*. Furthermore, the impression of fast population regeneration is based on rough estimates. Controlled measurements could enable appropriate adaptations of the control strategies.

Most pest ant species prefer tropical and subtropical climates and are not able to cope with frost

and lower temperatures as it used to be during winter months in Central Europe. The progression of global warming likely facilitates invasions to more northern latitudes. The pest ant species *F. fuscocinerea* can cope with temperatures below 0 °C and has the potential to spread in Central Europe. By now, *F. fuscocinerea* behaves problematically only in its native range. To prevent an unnoticed spreading process, it will be necessary to survey large abundances of *F. fuscocinerea* thoroughly.

5.2 Analyzing natural pheromone trails in *L. neglectus*

Another focus of this thesis was to better understand the competitive abilities of the invasive garden ant *L. neglectus*. A more differentiated pheromone communication in the invasive species is considered to particularly contribute to competitive advantages over closely related native species. Extractions of pheromone glands were offered to the ants in different experimental settings. The pheromone trail communication of the invasive garden ant *L. neglectus* is found to highly depend on hindgut pheromones. The use of pheromones glands in the invasive species did not differ from the native *L. niger* and *L. platythorax*. Various factors, however, remain unexplored. For example, this thesis did not account for varying pheromone concentrations in detail. At least two concentration levels were used in the pheromone trail experiments: lower pheromone concentrations when gland contents were solved for the use as artificial trails, and high pheromone concentrations when the whole glands were offered in the point-source experiment. Apart from that, no further concentration levels were tested in the pheromone experiments. Behavioral responses can differ depending on whether a high or a small amount of pheromone is released. This is particularly the case when pheromones with different glandular origins are mixed. Therefore, a next step could be analyzing the components and the concentrations of natural trail pheromones in detail. Due to the analysis of gland substances presented in Section 3.4 it is possible to determine the glands that are used for laying natural ant trails.

5.3 Developing species-specific control baits for *L. neglectus*

The results of this thesis suggests that hindgut trails are specific to the studied *Lasius* species. A main contribution of this thesis is the discovery of attractant 2,6-dimethyl-3-ethyl-5-hepten-1-ol, which appears to be specific to *L. neglectus*. In order to confirm species-specifity of this attractant, the experiments in this study need to be extended so as to exclude bioactivity on other non-target species, especially on those that are likely to co-exist with *L. neglectus*. The efficacy of this attractant as a poison bait has to be investigated. Furthermore, the analysis of natural pheromone trails may reveal alternative candidates worth to be examined.

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APPENDIX A

APPENDIX

A.1 Statistical background: PERMANOVA designs and additional results

The sections A.1.1 to A.1.4 are based on Pohl et al. (2018)

A.1.1 Permutational MANOVA: PERMANOVA

Frequently, statistical analyses of observations similar to some of those presented in this thesis involve (multivariate) analysis of variance, or (M)ANOVA. For (M)ANOVA to be applicable, data have to satisfy certain requirements (Anderson, 2001): The observations need to be independent from each other, the residuals of the data need to be normally distributed and the variance of data in groups should be the same. The data collected in this study were not normally distributed. Thus, for the majority of statistical significance tests non-parametric permutational multivariate analyses of variance (permutational MANOVA in the following called PERMANOVA (Anderson, 2001)) were used. PERMANOVA allows partitioning of variation across different factors, neither requiring multivariate normal distribution nor the use of Euclidean distance measures among multivariate groups (Anderson, 2001). The test statistic is an F-ratio. In the multivariate analysis the F-ratio does not follow the distribution of Fishers F-ratio under the null-hypothesis (Anderson, 2001). Thus, permutations of the observation units are used to create a simulated distribution under the null-hypothesis (Anderson, 2001). A re-sampling of observation units between groups results in new F-values (called $F\pi$) and, when repeated for all possible re-orderings, yields a pseudo-F-statistic under a true null-hypothesis for the particular data set (Anderson, 2001). The P-value is then calculated as

$$P = \frac{(\text{No. } F \geq F\pi)}{(\text{Total No. } F\pi)} \tag{A.1}$$

(Anderson, 2001). Especially in designs with more than one factor, not all observations are exchangeable under the null-hypothesis (Anderson, 2001). Restricting permutations, e.g. nesting of factors, is then necessary. If there is only one variable and if the Euclidean distance is used, the resulting sum of squares and F-ratios are the same as in Fishers univariate F-statistic in the traditional ANOVA (Anderson, 2001).

A.1.2 Field study

The test design of the PERMANOVA used for the analysis of the bait experiment comprised six factors, i.e. *date of observation*, *time of observation*, *section*, *bait station*, *bait type* and *sun exposure* at the bait stations. Two covariates were included, *temperature* at the bait stations and relative *humidity* for each observation time.

Table A.1: PERMANOVA design for the field study with *F. fuscocinerea*, *M. ruginodis* and *L. niger* in a natural habitat. Adapted from Pohl et al. (2018).

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Date of observation[1]	5		random
Time of observation[2]	7		random
Section[3]	4		random
Bait station[4]	35	section	random
Bait type[5]	2	bait station	fixed
Sun exposure[6]	3	section	fixed
Covariates			
Humidity[7]			
Temperatur[8]			

[1]Observations were made on five days: 28th/30th June, 2nd/6th/9th July; [2]observations were made seven times a day (at 8:30 a.m., 9:30 a.m., 10:30 a.m., 3:30 p.m., 4:30 p.m., 8:30 p.m., 9:30 p.m.); [3]the habitat was divided into four sections according to differences in substrate and vegetation; [4]thirty-five bait stations were observed; every bait station was assigned to one of the four habitat sections, hence *bait station* was nested in *section*; [5]every bait station contained two bait types: honey and tuna; every bait was assigned to a bait station, hence *bait type* was nested in *bait station*; [6]sun exposure categories: sunny, semi-shady and shady; sun exposure was assigned to one of the four habitat sections, hence *sun exposure* was nested in *section*; [7]relative air humidity was measured at one shady point in the habitat every time of observation; [8]temperature was measured at every bait station every time of observation;

A.1.3 Activity test

The PERMANOVA design for the activity test comprised three factors: *daytime, tree* and *direction* and all combinations of the factors (Table A.2).

Table A.2: Test design for the activity test with *F. fuscocinerea* in a natural habitat. Adapted from Pohl et al. (2018).

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Daytime[1]	2		fixed
Tree[2]	7		random
Direction[3]	2 (up, down)		fixed
Interactions			
Daytime × tree[4]			
Daytime × direction[5]			
Tree × direction[6]			

[1]Counts repeated at four different times were assigned to two daytimes: day (10:00 a.m., 3:00 p.m.), night (11:00 p.m., 12:00 a.m.); [2]foraging activity was observed on seven different spruce trunks; [3]ants were distinguished whether they go up or down the tree trunk. [4]analysis of interactions among factors: differences in ant numbers on different trees at different daytimes; [5]differences in ant numbers going up or down at different daytimes; [6]differences in ant numbers going up or down at different trees.

A.1.4 Exploitative and interference competition experiment

Table A.3: PERMANOVA design A) for the first part, B) for the second part of the exploitative and interference competition experiment with *F. fuscocinerea, M. ruginodis* and *L. niger*. Adapted from Pohl et al. (2018).

A)

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Explorer species[1]	3		fixed
Trial number	40	explorer species	random
Covariate			
Time			
Contrasts			
Name	**Factor**	**Contrast**	
C1	explorer species	*F. fuscocinerea* vs. *M. ruginodis*	
C2	explorer species	*F. fuscocinerea* vs. *L. niger*	
C3	explorer species	*M. ruginodis* vs. *L. niger*	
Interactions of factors			
Time × explorer species			

[1] *F. fuscocinerea, M. ruginodis, L. niger.*

B)

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Competitor species[1]	4		fixed
Trial number	40	competitor species	random
Covariate			
Time			
Contrasts			
Name	**Factor**	**Contrast**	
C1	competitor species	$F.\ fuscocinerea_{M.\ ruginodis}$ vs. $F.\ fuscocinerea_{L.\ niger}$	
C2	competitor species	$M.\ ruginodis_{F.\ fuscocinerea}$ vs. $F.\ fuscocinerea_{M.\ ruginodis}$	
C3	competitor species	$F.\ fuscocinerea_{L.\ niger}$ vs. $L.\ niger_{F.\ fuscocinerea}$	
C4	competitor species	$L.\ niger_{F.\ fuscocinerea}$ vs. $M.\ ruginodis_{F.\ fuscocinerea}$	
Interactions of factors			
Time × competitor species			

[1] $F.\ fuscocinerea_{L.\ niger}$, $F.\ fuscocinerea_{M.\ ruginodis}$, $M.\ ruginodis_{F.\ fuscocinerea}$, $L.\ niger_{F.\ fuscocinerea}$;
the respective explorer species is given as subscript.

Table A.4: PERMANOVA design for the comparison between the first and the second part of the exploitative and interference competition experiment with *F. fuscocinerea, M. ruginodis* and *L. niger*. Adapted from Pohl et al. (2018).

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Group[1]	7		fixed
Trial number	80	group	random
Covariate			
Time			
Contrasts			
Name	**Factor**	**Contrast**	
C1	group	*F. fuscocinerea* vs. *F. fuscocinerea*$_{M.\ ruginodis}$	
C2	group	*F. fuscocinerea* vs. *F. fuscocinerea*$_{L\ niger}$	
C3	group	*M. ruginodis* vs. *M. ruginodis*$_{F.\ fuscocinerea}$	
C4	group	*L. niger* vs. *L. niger*$_{F.\ fuscocinerea}$	
Interactions of factors			
Species × time			

[1] Explorer species: *F. fuscocinerea, M. ruginodis, L. niger*; Competitor species: *F. fuscocinerea*$_{L.\ niger}$, *F. fuscocinerea*$_{M.\ ruginodis}$, *M. ruginodis*$_{F.\ fuscocinerea}$, *L. niger*$_{F.\ fuscocinerea}$; the respective explorer species is given as subscript.

Table A.5: PERMANOVA design A) for the first part, B) for the second part of the exploitative and interference competition experiment with *L. neglectus, L. niger* and *L. platythorax*

A)

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Explorer species[1]	3		fixed
Trial number	80	explorer species	random

Covariate

Time

Contrasts

Name	**Factor**	**Contrast**
C1	explorer species	*L. neglectus* vs. *L. niger*
C2	explorer species	*L. neglectus* vs. *L. platythorax*
C3	explorer species	*L. niger* vs. *L. platythorax*

Interactions of factors

Time × explorer species

[1] *L. neglectus, L. niger* and *L. platythorax*.

B)

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Competitor species[1]	4		fixed
Trial number	80	competitor species	random

Covariate

Time

Contrasts

Name	**Factor**	**Contrast**
C1	competitor species	*L. niger*$_{L.\ neglectus}$ vs. *L. neglectus*$_{L.\ niger}$
C2	competitor species	*L. platythorax*$_{L.\ neglectus}$ vs. *L. neglectus*$_{L.\ platythorax}$
C3	competitor species	*L. niger*$_{L.\ neglectus}$ vs. *L. platythorax*$_{L.\ neglectus}$
C4	competitor species	*L. neglectus*$_{L.\ niger}$ vs. *L. neglectus*$_{L.\ platythorax}$

Interactions of factors

Time × competitor species

[1] *L. neglectus*$_{L.\ niger}$, *L. neglectus*$_{L.\ platythorax}$, *L. niger*$_{L.\ neglectus}$, *L. platythorax*$_{L.\ neglectus}$;
the respective explorer species is given as subscript.

Table A.6: PERMANOVA design for the comparison between the first and the second part of the exploitative and interference competition experiment with *L. neglectus, L. niger* and *L. platythorax*

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Group[1]	7		fixed
Trial number	160	group	random
Covariate			
Time			
Contrasts			
Name	**Factor**	**Contrast**	
C1	group	*L. neglectus* vs. *L. neglectus*$_{L.\ niger}$	
C2	group	*L. neglectus* vs. *L. neglectus*$_{,\ platythorax}$	
C3	group	*L. niger* vs. *L. niger*$_{L.\ neglectus}$	
C4	group	*L. platythorax* vs. *L. platythorax*$_{L.\ neglectus}$	
Interactions of factors			
Species × time			

[1]Explorer species: *L. neglectus, L. niger, L. platythorax*; competitor species: *L. neglectus*$_{L.\ niger}$, *L. neglectus*$_{L.\ platythorax}$, *L. niger*$_{L.\ neglectus}$, *L. platythorax*$_{L.\ neglectus}$; the respective explorer species is given as subscript.

A.1.5 One-on-one aggression tests with *L. neglectus, L. niger* and *L. platythorax*

Table A.7: One-on-one aggression tests with the invasive species *L. neglectus* and the native species, *L. niger* and *L. platythorax*. Kruskal-Wallis comparisons of the aggression indices of the species considering their opponent (shown as subscript). The table shows the number of observations (N), the test value (K) and the two-sided Monte-Carlo *P*-value. *P*-values less than 0.05 are marked in bold.

Species	**N**	**K**	*P*
L. neglectus$_{L.\ niger}$	15		
L. neglectus$_{L.\ platythorax}$	15		
L. neglectus$_{\text{nestmate control}}$	12		
L. niger$_{L.\ neglectus}$	15	29.093	**< 0.001**
L. niger$_{\text{nestmate control}}$	12		
L. platythorax$_{L.\ neglectus}$	15		
L. platythorax$_{\text{nestmate control}}$	12		

A.1.6 Aggression test with group of *Lasius* ants

Table A.8: PERMANOVA design for the aggression test with group of *Lasius* ants

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Species combination[1]	4		fixed

[1] *L. neglectus*$_{L.\ niger}$, *L. neglectus*$_{L.\ platythorax}$, *L. niger*$_{L.\ neglectus}$, *L. platythorax*$_{L.\ neglectus}$; the respective explorer species is given as subscript.

A.1.7 Pheromone trail communication

A.1.7.1 Direction-by-choice experiment

Table A.9: PERMANOVA designs for the direction-by-choice experiment

A)

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Species[1]	3		random
Gland type[2]	4		fixed
Interactions of factors			
Species × gland type			

B)

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Species	3		random
Gland type	4	species	fixed
Interactions of factors			
Species × gland type (species)			

C)

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Gland type	4		fixed
Species	3	gland type	random
Interactions of factors			
Gland type × species (gland type)			

[1] *L. neglectus*, *L. niger* and *L. platythorax*; [2] hindgut, mandibular gland, poison gland, Dufour's gland

Table A.11: Direction-by-choice experiment with the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*. PERMANOVA post-hoc pairwise comparisons of the species. Analyzed is the number of ants following the artificial gland trail, i.e. mandibular gland, hindgut, poison gland or Dufour's gland trails, on a Y-shaped bridge. The table shows the test statistics (t), *P*-values determined by permutations and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

Groups	**t**	***P* (perm)**	**Unique perms**
L. neglectus, *L. niger*	3.558	**< 0.001**	9925
L. neglectus, *L. platythorax*	1.3946	0.168	9795
L. niger, *L. platythorax*	1.9068	0.055	9929

Table A.10: Direction-by-choice experiment with the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*: PERMANOVA analysis of the number of ants following the artificial gland trail, i.e. mandibular gland, hindgut, poison gland or Dufour's gland trails, on a Y-shaped bridge. The PERMANOVA design took two factors (*species* and *gland*) into account. The table shows the degrees of freedom (df), sums of squares (SS), mean squares (MS), Pseudo-*F* values, *P*-values determined by permutations and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

Source	**df**	**SS**	**MS**	**Pseudo-*F***	***P* (perm)**	**Unique perms**
Species[1]	2	2871.1	1435.6	5.876	**0.005**	9944
Gland type[2]	3	49754	16585	14.858	**0.006**	9975
Species × gland type	4	6701.1	1116.8	4.5714	**0.001**	9935
Residual	243	59368	244.31			
Total	254	1.2003E5				

[1] *L. neglectus*, *L. niger* and *L. platythorax*; [2] hindgut, mandibular gland, poison gland and Dufour's gland

A.1.7.2 Accuracy-in-trail-following experiment

Table A.12: PERMANOVA design for the accuracy-in-trail-follwing experiment

A)

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Test nest[1]	9/8/6		random
Gland type[2]	6		fixed
Trail section[3]	4		random
Direction[4]	2		random
Interactions of factors			
Test nest × gland type			
Test nest × trail section			
Test nest × direction			
Trail section × direction			

B)

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Test nest	9/8/6		random
Trail section	4		random
Gland type	6	trail section	fixed
Direction	2	gland type	random
Interactions of factors			
Test nest × gland type (trail section)			
Test nest × trail section			
Test nest × direction (gland type (trail section)))			
Trail section × direction (gland type (trail section)))			

C)

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Test nest	9/8/6		random
Gland type	6		fixed
Trail section	4	gland type	random
Direction	2	gland type	random
Interactions of factors			
Test nest × gland type			
Test nest × trail section (gland type)			
Test nest × direction (gland type)			
Trail section (gland type) × direction (gland type)			

[1] 9 *L. neglectus* nests, 8 *L. niger* nest and 6 *L. platythorax* nest were tested; [2] hindgut, mandibular gland, poison gland, Dufour's gland, DCM control, pencil control; [3] to analyze the accuracy of trail following of the ants in dependence of trail length, the trail was divided into four sections: the first quarter of the trail length, the first half of the trail length, the first 3/4 of the trail length and the entire trail length; [4] two directions were analyzed: from the nest to the food source, from the food source to the nest.

Table A.13: Accuracy-in-trail-following experiment with the invasive *L. neglectus* and the native species, *L. niger* and *L. platythorax*: PERMANOVA analyses with ant numbers following an artificial S-shaped gland trail which is divided into four sections. The PERMANOVA design took four factors (*testnest, gland, trailsection* and *direction*) and the interaction between factors into account. The table shows the degrees of freedom (df), sums of squares (SS), mean squares (MS), Pseudo-*F* values, *P*-values determined by permutations and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

A) *L. neglectus*

Source	**df**	**SS**	**MS**	**Pseudo-*F***	***P* (perm)**	**Unique perms**
Test nest	8	1.5194	0.18993	0.93946	0.074	9858
Gland type	5	33.711	6.7422	2.751	**0.004**	9929
Trail section (gland type)	18	28.891	1.605	9.8001	**< 0.001**	9856
Direction (gland type)	6	3.4199	0.56998	2.1862	**0.004**	9886
Test nest × gland	34	5.5124	0.16213	0.84329	**0.035**	9779
Test nest × trail section (gland type)	126	10.005	7.9408E-2	0.67992	0.998	9736
Test nest × direction (gland type)	17	4.0865	0.24038	2.0582	**0.003**	9901
Trail section (gland t.) × direction (gland t.)	18	1.4527	8.0704E-2	0.69102	0.894	9886
Residual	307	35.855	0.11679			
Total	539	144.58				

B) *L. niger*

Source	**df**	**SS**	**MS**	**Pseudo-*F***	***P* (perm)**	**Unique perms**
Test nest	7	4.9609	0.7087	1.4449	**0.004**	9861
Gland type	5	43.833	8.7666	4.9819	**< 0.001**	9946
Trail section (gland type)	18	14.427	0.80153	4.0335	**< 0.001**	9750
Direction (gland type)	6	1.1953	0.19922	0.68026	0.211	9856
Test nest × gland type	35	16.02	0.45772	1.0215	**0.004**	9795
Test nest × trail section (gland type)	126	13.416	0.10647	0.77745	0.987	9768
Test nest × direction (gland type)	6	2.4411	0.40686	2.9708	**0.003**	9931
Trail section (gland t.) × direction (gland t.)	18	1.3806	7.6698E-2	0.56004	0.981	9879
Residual	286	39.168	0.13695			
Total	507	155.53				

C) *L. platythorax*

Source	**df**	**SS**	**MS**	**Pseudo-*F***	***P* (perm)**	**Unique perms**
Test nest	5	3.8046	0.76092	2.8704	**< 0.001**	9887
Gland type	5	36.588	7.3175	3.6228	**< 0.001**	9897
Trail section (gland type)	18	13.04	0.72445	3.9728	**< 0.001**	9848
Direction (gland type)	6	7.6394	1.2732	3.2386	**< 0.001**	9874
Test nest × gland type	19	7.1504	0.37634	1.4529	**< 0.001**	9763
Test nest × trail section (gland type)	72	6.4578	8.9692E-2	0.71183	0.988	9802
Test nest × direction (gland type)	18	4.9751	0.27639	2.1936	**< 0.001**	9860
Trail section (gland t.) × direction (gland t.)	18	2.9596	0.16442	1.3049	0.124	9895
Residual	346	43.597	0.126			
Total	507	152.17				

Table A.14: PERMANOVA design for the accuracy-in-trail-follwing experiment

A)

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Trail section[1]	4		fixed
Gland type[2]	6	trail section	random
Species[3]	3	gland type	random

[1]To analyze the accuracy of trail following of the ants in dependence of trail length, the trail was divided into four sections: the first quarter of the trail length, the first half of the trail length, the first 3/4 of the trail length and the entire trail length; [2]to compare trail sections within the respective gland type (i.e. mandibular gland, hindgut, poison gland and Dufour's gland) the factor *gland* was nested in the factor *trail section*; [3]to compare the species within the respective trail section within the respective gland the factor *species* was nested the factor *gland*.

Table A.15: Accurarcy-in-trail-following experiment with the invasive *L. neglectus* and the native species, *L. niger* and *L. platythorax*: PERMANOVA analyses with ant numbers following an artificial S-shaped gland trail which is divided into four sections. The PERMANOVA design took three factors (*trail section*, *gland*, *species* into account. The table shows the degrees of freedom (df), sums of squares (SS), mean squares (MS), Pseudo-F values, P-values determined by permutations and the numbers of unique permutations. P-values less than 0.05 are marked in bold.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P (perm)**	**Unique perms**
Trail section[1]	3	43.414	14.471	1.784	0.157	9950
Gland type (trail section)[2]	20	162.29	8.1147	11.615	**< 0.001**	9915
Species (gland type (trail section))[3]	48	33.583	0.69965	4.7144	**< 0.001**	9818
Residual	1484	220.24	0.14841			
Total	1555	460.13				

[1]To analyze the accurarcy of trail following of the ants in dependence of trail length, the trail was divided into four sections: the first quarter of the trail length, the first half of the trail length, the first 3/4 of the trail length and the entire trail length; [2]to compare trail sections within the respective gland type (i.e. mandibular gland, hindgut, poison gland and Dufour's gland) the factor *gland* was nested in the factor *trail section*; [3]to compare the species within the respective trail section within the respective gland the factor *species* was nested the factor *gland* which was nested in the factor *trail section*.

A.1.7.3 Alternative-trail-branch experiment

Table A.16: PERMANOVA designs for the alternative-trail-branch experiment

A)

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Species[1]	3		random
Gland type[2]	5		fixed
Interactions of factors			
Species × gland type			

B)

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Species	3		random
Gland type	5	species	fixed
Interactions of factors			
Species × gland type (species)			

C)

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Gland type	5		fixed
Species	3	gland type	random
Interactions of factors			
Gland type × species (gland type)			

[1] *L. neglectus*, *L. niger* and *L. platythorax*; [2] hindgut, mandibular gland, poison gland, Dufour's gland and DCM control

Table A.17: Alternative-trail-branch experiment with the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*. PERMANOVA analyses with number of ants turning to the artificial gland trail. The PERMANOVA design took two factors (*species* and *gland*) and the interaction between *species* and *gland* into account. The table shows the degrees of freedom (df), sums of squares (SS), mean squares (MS), Pseudo-F values, P-values determined by permutations and the numbers of unique permutations. P-values less than 0.05 are marked in bold.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P (perm)**	**Unique perms**
Species[1]	2	12.098	6.049	33.104	**0.039**	9952
Gland[2]	4	370.04	92.511	70.626	**< 0.001**	9939
Species x Gland	8	10.479	13.098	0.71684	0.678	9931
Residual	286	522.59	18.272			
Total	300	915.09				

[1] *L. neglectus*, *L. niger* and *L. platythorax*; [2] hindgut, mandibular gland, poison gland, Dufour's gland and DCM control

Table A.18: Alternative-trail-branch experiment with the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*. PERMANOVA post-hoc pairwise comparisons of the species. Analyzed is the number of ants turning to the artificial gland trail, i.e. mandibular gland, hindgut, poison gland or Dufour's gland trails. The table shows the test statistics (t), *P*-values determined by permutations and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

Groups	**t**	***P* (perm)**	**Unique perms**
L. neglectus, *L. niger*	0.10797	0.910	9793
L. neglectus, *L. platythorax*	20.491	**0.043**	9838
L. niger, *L. platythorax*	23,528	**0.018**	9814

A.1.7.4 Single-point-source experiment

Table A.19: PERMANOVA design for the single-point-source experiment

A)

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Species[1]	3		fixed
Gland type[2]	5	species	random

[1] *L. neglectus*, *L. niger* and *L. platythorax*; [2]hindgut, mandibular gland, poison gland, Dufour's gland and DCM control

Table A.20: Single-point-source experiment with the invasive *L. neglectus* and the native, *L. niger* and *L. platythorax*. PERMANOVA analyses of the number of different behaviors the ants showed when passing the gland source. The PERMANOVA design took two factors (*species* and *gland*) and the interaction between *species* and *gland* into account. The table shows the degrees of freedom (df), sums of squares (SS), mean squares (MS), Pseudo-*F* values, *P*-values determined by permutations and the numbers of unique permutations. *P*-values less than 0.05 are marked in bold.

Source	**df**	**SS**	**MS**	**Pseudo-*F***	***P* (perm)**	**Unique perms**
Species[1]	2	10.799	5.3995	4.9213	**0.019**	9955
Gland type (species)[2]	12	13.198	1.0998	13.191	**< 0.001**	9892
Residual	281	23.429	8.3378E-2			
Total	295	46.71				

[1] *L. neglectus*, *L. niger* and *L. platythorax*; [2]hindgut, mandibular gland, poison gland, Dufour's gland and DCM control

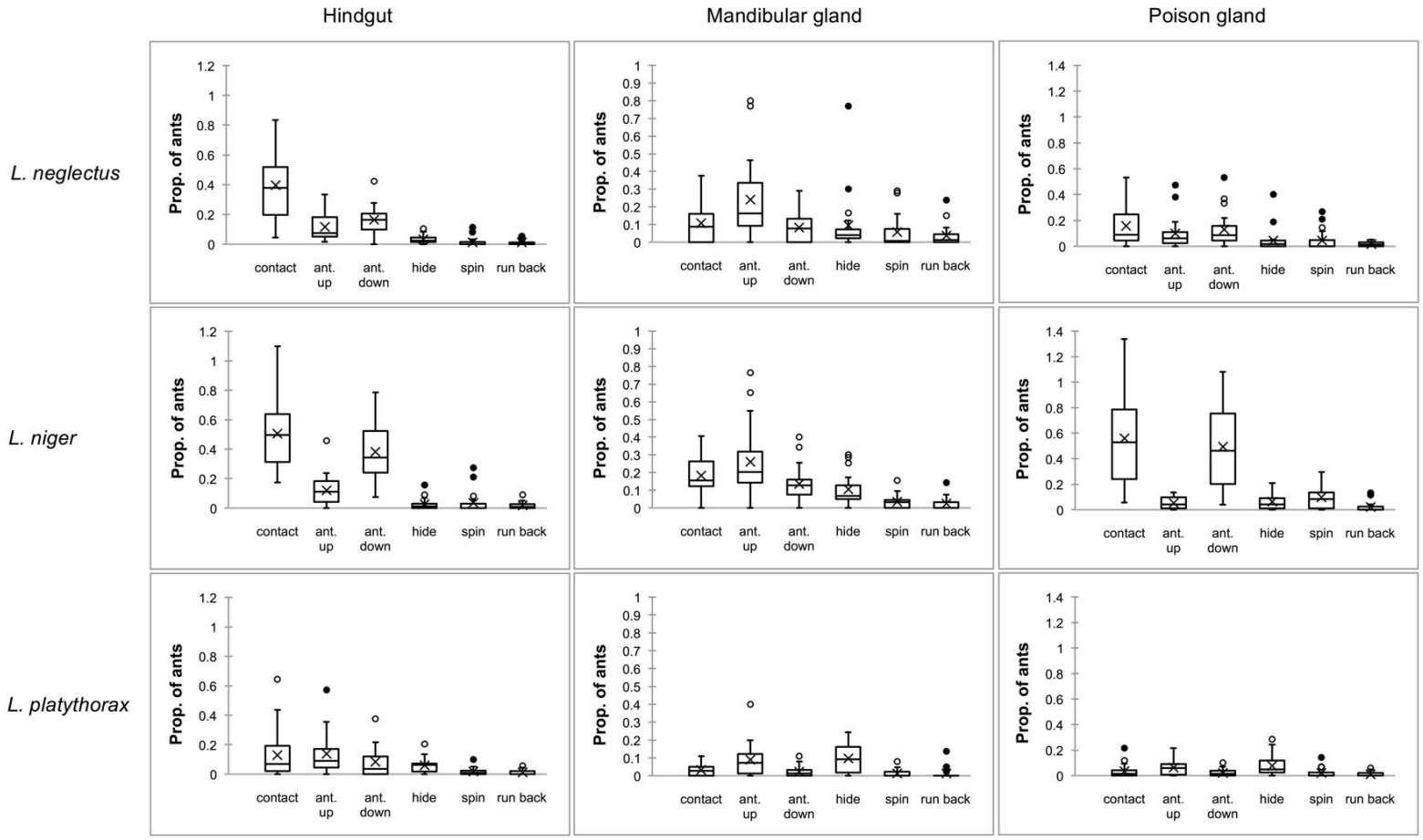

Figure A.1: Single-point-source experiment with the invasive *L. neglectus* and the native *L. niger* and *L. platythorax*. Proportion of ants that showed a certain behavior when they encountered a squashed hindgut, mandibular gland or poison gland next to their natural pheromone trail. Box-and-whisker plots with first and third quartiles, median and whiskers (minimum and maximum value); mean (×); values that are more than 1.5 times the interquartile range (IQR) are displayed as outliers (○), values that are more than 3 IQR are displayed as extreme points (•).

Figure A.2: Single-point-source experiment with the invasive *L. neglectus* and the native *L. niger* and *L. platythorax*. Proportion of ants that showed a certain behavior when they encountered a squashed Dufour's gland or the control treatment next to their natural pheromone trail. Box-and-whisker plots with first and third quartiles, median and whiskers (minimum and maximum value); mean (×); values that are more than 1.5 times the interquartile range (IQR) are displayed as outliers (∘), values that are more than 3 IQR are displayed as extreme points (•).

A.1.8 Analyses of the gland substances

Table A.21: PERMANOVA design for the comparison of gland substances of *L. neglectus*, *L. niger* and *L. platythorax*.

Factor	**Nr. of levels**	**Nested in**	**random/fixed**
Comparison	18		fixed
Contrasts			
Name	**Factor**	**Contrast**	
C1	comparison	(LN[1]_D[2](C[3])) vs. (LN_D)	
C2	comparison	(LN_P[2](C)) vs. (LN_P)	
C3	comparison	(LN_H[2](C)) vs. (LN_H)	
C4	comparison	(LNE[1]_D(C)) vs. (LNE_D)	
C5	comparison	(LNE_P(C)) vs. (LNE_P)	
C6	comparison	(LNE_H(C)) vs. (LNE_H)	
C7	comparison	(LP[1]_D(C)) vs. (LP_D)	
C8	comparison	(LP_P(C)) vs. (LP_P)	
C9	comparison	(LP_H(C)) vs. (LP_H)	
C10	comparison	(LN_D) vs. (LNE_D)	
C11	comparison	(LN_D) vs. (LP_D)	
C12	comparison	(LNE_D) vs. (LP_D)	
C13	comparison	(LN_P) vs. (LNE_P)	
C14	comparison	(LN_P) vs. (LP_P)	
C15	comparison	(LNE_P) vs. (LP_P)	
C16	comparison	(LN_H) vs. (LNE_H)	
C17	comparison	(LN_H) vs. (LP_H)	
C18	comparison	(LNE_H) vs. (LP_H)	

[1]Species names abbreviations: LNE: *L. neglectus*, LN: *L. niger*, LP: *L. platythorax*; [2]pheromone gland abbreviations: H: hindgut and rectum, P: poison gland, D: Dufour's gland; [3]control treatment for the respective pheromone gland;