The influence of the symbiotic fungus on foraging decisions in leaf-cutting ants

Individual behavior and collective patterns

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GENERAL INTRODUCTION

Leaf-cutting ants are of immense importance in tropical and subtropical ecosystems occurring all over South and Central America and in parts of the southern United States. Their colony sizes range from only several thousands of workers in the genus *Acromyrmex* up to several millions of individuals in mature *Atta* colonies (Fowler et al. 1986) and they harvest between 85 and 470 kg (dry weight) total plant bio mass per year (Hölldobler & Wilson 2008). Harvesting and processing such enormous amounts of plant material needed to culture their symbiotic fungus is only possible through division of labor and communication among the many individuals in a colony. As can be expected whenever two kinds of organisms live in close mutualistic symbiosis, communication is also a vital part in the ant-fungus relationship.

Leaf-cutting ants are highly polyphagic (Cherrett 1989; Wirth et al. 2003) and their ability to exploit a broad variety of plant species is a key issue in their ecological success. The ants are able to overcome mechanical plant defenses and their symbiotic fungus can cope with chemical defenses. This is only possible through the intricate interplay between the ants and their symbiont as the harvested plant material brought back to the nest is used to culture the fungus which in return provides the ants with food for their developing brood and larvae. Suitability of harvested substrate is therefore evaluated twice, once by foragers in the field and a second time through the fungus inside the nest. Once the substrate has been brought back to the nest, the specific tasks necessary during the complex process of preparation and incorporation of the substrate into the fungus are carried out according to the body size of the workers (Weber, 1966; Wilson 1980). Leaf fragments are subsequently inoculated with fungal cultivar (Mangone & Currie, 2007; Herz et al. 2008) and further tended (Bass & Cherrett, 1994, 1996) by the gardening ants.

If the substrate proves to be unsuitable for the fungus due to secondary compounds, foragers adjust their foraging behavior accordingly, i.e. stop harvesting said substrate (Ridley et al. 1996; North et al. 1999). Such delayed rejection implies avoidance learning by the foragers. The phenomenon of delayed rejection has already been shown in several leaf-cutting ant species (*Ac. lundi, Ac. octospinosus, Ac. subterraneus, A. cephalotes, A. laevigata, A. sexdens, A. colombica*) both in the laboratory (Knapp et al. 1990; Ridley et al. 1996; North et al. 1999; Camargo et al. 2003; Herz et al. 2008) and in the field (Ridley et al. 1996; Wagner 2004; Saverschek et al. 2010). Delayed rejection occurs species specific and within 24 hours (Herz et al. 2008; Saverschek et al. 2010) even though mature colonies usually harvest several plant species simultaneously and leaf fragments are transferred several times from one individual to the next from the cutting site until the final stages of processing inside the nest.

Due to the colony's size, the number of individuals, the low trail fidelity (15-45 %, Porter and Bowers 1982, Wagner 2004) and the time delay until detrimental effects on the fungus become detectable after the incorporation of unsuitable substrate, ants foraging on the same trail might have different experiential backgrounds regarding certain substrates. It therefore seems conceivable that information about plant suitability might be communicated not only inside the nest, but also outside on the trails throughout the harvesting process. Nonetheless, recognition of unsuitable substrate inside the nest is of key importance in the regulation of foraging activity as there the association between substrate and fungus performance is formed.

The aim of this study is to unravel the factors involved in the rejection of plant material unsuitable for the symbiotic fungus. In order to gain a better understanding of the processes involved, I analyze factors influencing decision making both inside and outside the colony, looking at collective patterns as well as individual behavior.

In order to learn about the unsuitability of a plant species, workers need to be able to identify incorporated plant species and associate them with detrimental effects on the fungus locally (Herz et al. 2008, personal communication). In chapter 1 of my thesis, I first address the question if plant odor is sufficient to distinguish between different plant species and if it might be a main characteristic to recall associations formed inside the nest out in the field during foraging. Many studies have looked at influences on plant preferences of individuals during the foraging cycle, but few have focused on information flow inside the nest. Here, workers can gain information about the suitability of the harvested substrate not only directly from the fungus, but maybe also indirectly through other workers. Even though the existence of feedback from the fungus has been proven and a lot is known about the general time frame of rejection, little is known about the mechanisms underlying the observed patterns. In chapter 2, I conduct several experimental series to elucidate under which conditions foragers without own foraging experience can learn about substrate suitability inside the nest separating the influence from the fungus from the influence of experienced gardeners or experienced foragers on nestmates without previous negative encounter with this substrate. From the collective level, I move on to a more detailed analysis of the individual behavior of foragers and gardeners inside the nest in chapter 3 that might explain how the observed pattern emerged.

As foragers spend a considerable amount of time outside the nest on trails throughout their daily foraging bouts (Shepherd 1982), it seems conceivable that information about plant suitability might be communicated not only inside the nest, but also outside on the trails throughout the harvesting process. Variation in plant preferences among workers creates an environment in which social information might be a factor influencing foraging decisions of individuals. The question arises if individuals foraging in a group make different, maybe more accurate foraging decisions due to social information available on the way to the food source than individuals foraging on their own (chapter 4). In other words, does the presence of nestmates, inadvertently through cues or directly through signals, influence foragers on the trail resulting in an emergent foraging pattern of the colony that is more than the sum of its parts?

That foragers were positively influenced in their decisions through encounters with laden nestmates returning to the nest on the trail has already been shown. Workers of *Acromyrmex lundi* are influenced in their choice at a newly discovered food source through the odor of the fragment carried by a scout worker (Roces 1990) and recruited workers of *Atta colombica* clearly preferred resources encountered on the trail during their outward journey (Howard et al. 1996). Chapter 5 tries to elucidate the questions if preferences of foragers, without previous experience of the unsuitable substrate, are influenced through the presence or absence of experienced foragers on the trail during foraging. As foragers frequently return to the nest between foraging trips, the situation inside the fungus chambers also needed to be put into consideration. After investigating the influence of the presence or absence of experienced foragers on the acceptance of substrate by foragers without previous experience on a collective level, I have a closer look on individual interactions in chapter 6. A laden recruit's behavior on the way back to the nest and its interactions with nestmates depending on the perceived suitability of its load are analyzed to elucidate possible mechanism explaining information transfer between foragers on the trail.

Much of the ants' social behavior is shaped by the details of the symbiotic relationship, whether it is though feedback about plant suitability from the fungus directly or indirectly through the need for harvesting and processing enormous amounts of leaf material and the foraging effort it involves. Surprisingly, to date, only few studies have examined the possibility of communication between the fungus and the ants.

CHAPTER 1

OLFACTORY MEMORY UNDERLIES DELAYED AVOIDANCE OF PLANTS UNSUITABLE FOR THE SYMBIOTIC FUNGUS

Abstract

Foraging decisions of leaf-cutting ant colonies are the result of an intricate interplay between workers and their symbiotic fungus. Plant species initially accepted by foragers might be later rejected if the substrate proves to be unsuitable for the fungus. To learn about the substrates unsuitability, workers need to be able to identify the incorporated plant species and associate it with the detrimental effects on the fungus. Odor is an important plant characteristic known to be used as a recognition key outside the nest in the context of foraging. This study shows that foragers of *Acromyrmex ambiguus* were able to learn about a plant's unsuitability for their symbiotic fungus and rejected this substrate in a binary choice experiment. Presented with leaf disks of two plant species simultaneously, one known as unsuitable and one as suitable, individual foragers significantly preferred the suitable plant species. When only presented with the plant odor, foragers steered away from the odor of the unsuitable plant species, thus avoiding it, moving towards the simultaneously presented odor of a suitable plant species. Foragers were therefore able to identify plant species and recall information about substrate suitability from the fungus through odor alone. Nonetheless, foragers showed a significantly stronger rejection towards unsuitable substrate when they could contact the leaf disks and therefore evaluate all leaf characteristics to identify plant species. It still remains unclear how ants learn about a plant's unsuitability from the fungus, but the ability of leaf-cutting ants to distinguish between two simultaneously presented plant odors outside the nest and their ability to recall information about a plant's unsuitability based on odor alone leads to the conclusion that inside the nest, odor might be enough to identify an incorporated substrate.

Introduction

Leaf-cutting ants live in habitats with highly diverse plant communities and a key issue in their ecological success is the ability to exploit a broad variety of plant species. They harvest 50 - 80 % of the occurring plant species, usually several plant species simultaneously (Cherrett 1989; Wirth et al. 2003). Their symbiotic fungus plays a role in shaping the foraging pattern of a colony through its feedback to foragers about plant suitability. Out in the field, foragers decide on plant material for their symbiotic fungus based on innate tendencies and experience. A second level of quality control occurs inside the nest, in the fungus gardens. Physical plant characteristics like hardness evaluated by foragers throughout processing can lead to post-selection of plant material (Camargo et al. 2003) and, more importantly, if the substrate proves to be unsuitable for the fungus due to chemical components, foragers adjust their foraging behavior accordingly, i.e. stop harvesting said substrate (Ridley et al. 1996; North et al. 1999). Such delayed rejection implies avoidance learning by the foragers.

The phenomenon of delayed rejection of substrate has already been shown in several leaf-cutting ant species (*Ac. lundi, Ac. octospinosus, Ac. subterraneus, A. cephalotes, A. laevigata, A. sexdens, A. colombica*) both in the laboratory (Knapp et al. 1990; Ridley et al. 1996; North et al. 1999; Camargo et al. 2003; Herz et al. 2008) and in the field (Ridley et al. 1996; Saverschek et al. 2010). It occurs very fast, within 24 hours, and is species specific (Herz et al. 2008; Saverschek et al. 2010). Plant identification is therefore of utmost importance out in the field as well as inside the nest. Epicuticular waxes, trichomes, leaf toughness or water content are regarded as main factors for the identification of substrates at the foraging site (Howard 1987, 1988; Cherrett 1989; Nichols-Orians & Schultz 1990), but it is unknown which of these are still available after the incorporation of the substrate into the fungus.

It is already known that the smell of harvested substrate can either serve as a mere orientation cue, where foragers walk against the wind towards the odor until they find the substrate (*Atta* and *Acromyrmex*, Littledyke & Cherrett 1978; *Cataglyphis*, Wolf & Wehner 2000, 2005) or as a conditioned cue used as decision criterion during substrate collection (*Acromyrmex lundi*, Roces 1990, 1994; *Camponotus mus*, Provecho & Josens 2009). In leaf-cutting ants, odor could play a particularly important role as a key characteristic in plant identification, not only outside, but also inside the nest.

We addressed the question if odor of an unsuitable plant species would be sufficient to recall the negative association foragers had made with this plant after its incorporation into the fungus and subsequently cause foragers to reject the plant odor during foraging due to their previous experience. To answer this question, we took advantage of the phenomenon of delayed rejection by testing individual foragers of *Acromyrmex ambiguus* in a binary choice experiment before and after the incorporation of unsuitable substrate into the fungus. As a prerequisite individuals were simultaneously offered leaf-disks of two plant species and their preferences before and after the incorporation of the unsuitable substrate was tested. In the next step foragers' ability to identify and therefore avoid substrate that had proven to be unsuitable for the fungus based on the odors of both plant species alone was tested.

Methods

Individual foragers' plant preferences were tested in binary choice experiments in two different experimental set-ups. First, as a prerequisite, foragers could choose between leaf disks of the two tested plant species. In the second experimental set-up, the odour set-up, foragers had to decide between two plant species based solely on the plant odours.

Subcolonies

Experiments were conducted with subcolonies obtained from 5 large lab colonies of *Acromyrmex ambiguus*. The colonies were collected in Uruguay in 2002 and maintained in the laboratory at 25°C and under a LD cycle of 12:12 h. Artificial nests consisted of three transparent Plexiglas®-boxes connected by short PVC-tubes (10 cm, Ø 3 cm). The center box (19 x 8.5 x 8.5 cm) served as the fungus chamber and the two other boxes (19 x 19 x 8.5 cm) as feeding-box and refuse-box respectively. The bottom of the fungus-box was covered with moistened expanded clay pebbles to keep the humidity high and prevent desiccation of the fungus. The lids of the refuse- and feeding-boxes had three holes each (Ø 3 cm), covered with a fine metallic mesh allowing air circulation to create a different, dryer microclimate from the one inside the fungus-box, an *inside* and *outside* for the subcolony.

About 1000 ml of fungus garden (fungus and gardening workers) were taken from the mother colony and placed in the artificial nest together with approximately 1000 foragers. Subcolonies were established at least 4 days prior to the start of the experiment to ensure well established fungus gardens and active foraging behavior. Subcolonies received fresh rose leaves (*Rosa canina*) and water every day and honey water every other day.

Experimental set-ups

The feeding-box of the subcolony was disconnected and replaced with a PVC-tube leading directly from the fungus chamber to the top of the entry platform (Figure 1.1). From this platform, a 5 cm wide and 1.5 m long wooden bridge (*main trail*) lead to another box (19 x 19 x 8.5 cm) used as experimental feeding arena. A movable side element, the *transfer bridge*, (10 x 0.5 cm) could be connected to the *main trail* halfway between the standard feeding arena and the experimental feeding arena. The transfer bridge led to the experimental set-up.

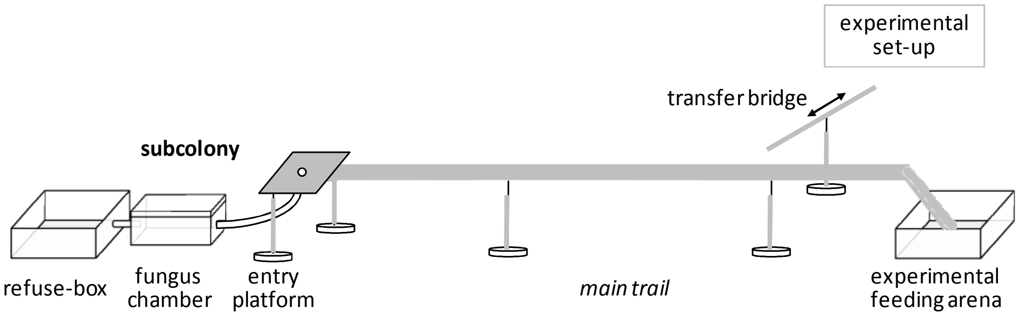

Figure 1.1: General set-up. A wooden bridge connected the entry platform with the experimental feeding arena 1.5 m away. The transfer bridge to single out individual foragers to the experimental set-up was placed 1 m away from the entry platform and 50 cm away from the experimental feeding arena.

The ***leaf set-up*** consisted of a rectangular platform (2 x 4 cm) with a toothpick (3 cm) attached in the center of the long side (Figure 1.2, *left*). One leaf disk of each tested plant species was placed on the platform equidistantly from the entering point via the toothpick.

The ***odor set-up*** consisted of two wooden squares (10 x 10 cm) attached to each other at a 90° angle (Figure 1.2, *right*). A short needle to pin three leaf disks was glued in the center of each square and a toothpick (1.5 cm) was attached 2 cm below. A third toothpick (3 cm) was placed in front of the two others, leaving a 5 mm gap.

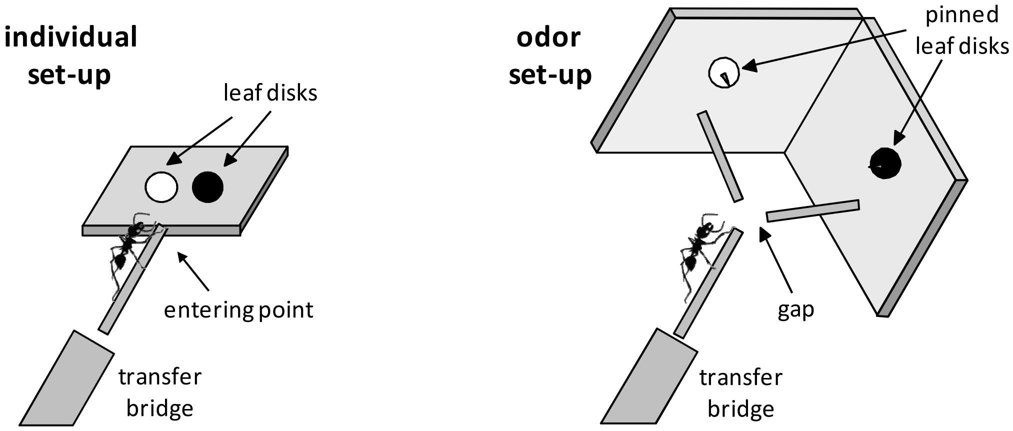

Figure 1.2: Experimental set-ups to test individual foragers. Leaf set-up (*left*): individual foragers were led towards a platform (2 x 4 cm) with the two tested plant species offered as leaf disks equidistantly from the entering point of the platform. Odor set-up (*right*): individual foragers approached the set-up on a toothpick which led to a bifurcation leading in the direction of the two tested plant species.

Experimental series

Two experimental series were conducted with each set-up. First, two plant species known as suitable to the subcolonies, blackberry and plum, were offered in the control series. Afterwards, subcolonies received leaf disks of the preferred plant species treated with fungicide to create an unsuitable substrate known to the workers of the subcolony. In the treatment series conducted 24 hours later, one plant species was now known as unsuitable, whereas the other plant species was known as suitable to the subcolonies. The whole procedure was repeated in an independent assay and different subcolonies with a second plant pair, privet and blackberry.

Treatment of plant material: In order to change the suitability of the plant species, an aqueous solution of cycloheximide (CHX; Sigma-Aldrich®, Deisenhofen, Germany) was used. This fungicide is undetectable by the ants but has been proven to be a potent fungicide to the attine fungus in the laboratory (Ridley et al. 1996; North et al. 1999; Herz et al. 2008). In order to maintain leaf specific properties such as odor, surface characteristics and toughness, the leaf internal airspace was infiltrated with an aqueous solution (0.03 %, w/w) of cycloheximide (see Herz et al. 2008). Each subcolony received 110 leaf disks of the preferred plant species from the control series.

Experimental procedure

To guarantee well established foraging, subcolonies foraged along the main trail for at least 2 days previous to the experiment. They only received fresh rose leaves in the experimental feeding arena before and during experiments. On the day of the experiment, the colony was given time (between 20-30 min) to establish the foraging process along the main foraging trail, until a constant number of laden foragers returned from the feeding arena. During this time period, the transfer bridge was placed on the main trail to ensure pheromone markings on it. Pheromone markings increased the percentage of ants leaving the main trail and walking along the transfer bridge.

The transfer bridge was used to single out foragers from the *main trail*. Once a forager had entered the bridge, it was carefully moved towards the experimental set-up leaving a gap of several centimeters towards the main trail so no other forager could follow. In the ***leaf experiment***, the forager entered the platform via the toothpick encountering a leaf disk of each of the two tested plant species on it. The foragers' decision was recorded when it left the platform with one of the leaf disks. In the ***odor experiment***, the forager had to climb across the gap at the bifurcation (5 mm) to move towards one of the perceived plant odors. Crossing the gap in one direction or the other was recorded as decision for one of the two plant species presented. In between foragers, the platform in the leaf set-up and all toothpicks in the odor set-up were replaced to avoid any influence through potential pheromone traces. The sides on which the two plant species were presented were alternated to account for possible side preferences of the foragers. If an individual did not make a decision within 2 min, the test was discontinued and the next forager was tested. Leaf disks (Ø 6 mm) were punched out freshly and replaced after each forager.

Data analysis

Data of 40 successfully tested individuals, i.e. foragers that made a choice in the experimental set-up, was collected per subcolony with 8 subcolonies per experimental series. To comply with requirements of normality all values were arcsine transformed prior to statistical analyses. Significance of difference in acceptance from random distribution (50:50) was tested with t-test for single mean. Significant differences in acceptance between control and treatment in both set-ups were tested with paired t-tests. Decrease in acceptance was calculated as follows: difference in acceptance between before (control) and after the treatment as percentage of control. Values complied with the requirements of normality and unpaired t-tests were used to compare decrease in acceptance between the leaf and odour set-up in both plant pairs.

Results

Plant pairs were tested before (control) and after the treatment of one of the plant species with fungicide (treatment) in both experimental set-ups.

When foragers chose between leaf disks of blackberry and plum (Figure 1.3, *top left*), foragers significantly preferred blackberry in the control series when both plant species were suitable (tested against random distribution of 50:50; $p = 0.02$, $t = 3.19$, $df = 7$). After the treatment with fungicidal blackberry leaves, significantly less foragers accepted blackberry leaf disks 24 hours later compared to the control series ($p < 0.001$, $t = 7.50$, $df = 7$).

The same pattern could be found when foragers were only presented with leaf odors (Figure 1.3, *top right*). In the control series, when both plant species were known as suitable, a significantly higher percentage of foragers chose blackberry odor over plum odor ($p = 0.01$, $t = 3.25$, $df = 7$). In the treatment series 24 hours after the intake of fungicidal blackberry leaves, the previously preferred blackberry odor was chosen significantly less than in the control series ($p < 0.001$, $t = 9.70$, $df = 7$).

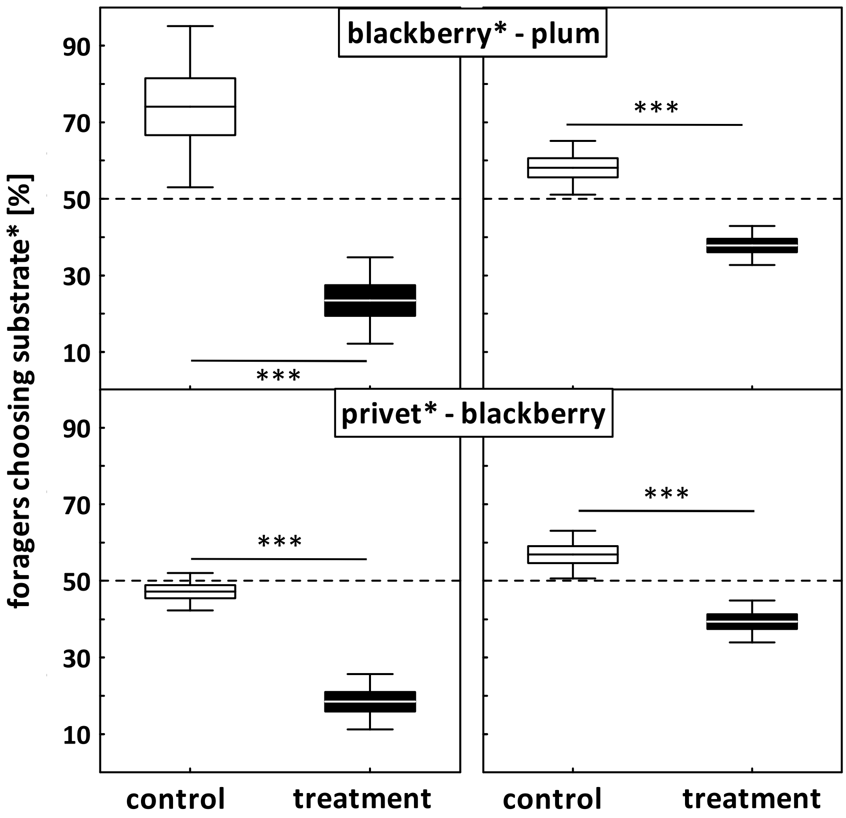

Figure 1.3: Comparison of preferences between control (☐) and treatment (■) in both experimental set-ups. Individuals were either exposed to leaf disks (*left*) or odours (*right*) of plants offered in pairs. Tested plant pairs: blackberry* - plum (*top*) and privet* - blackberry (*bottom*). Treated plant species within each pair are marked with an asterisk. Data are mean ± SE, and n = 8 subcolonies for each group (N = 40 individuals tested per subcolony). Significance of paired t-tests (control - treatment) is given as ***$p \leq 0.001$.

In the second plant pair tested, privet versus blackberry, the same pattern could be observed (Figure 1.3, *bottom*). In the leaf set-up foragers accepted privet and blackberry disks equally well in the control series ($p = 0.15$, $t = -1.62$, $df = 7$) and acceptance of privet had significantly decreased in the treatment series 24 hours later ($p < 0.001$, $t = 10.76$, $df = 7$). When foragers chose between odors of the two offered plant species, privet was significantly preferred in the control series and ($p = 0.02$, $t = 3.07$, $df = 7$) acceptance had significantly decreased in the treatment series ($p < 0.001$, $t = 8.14$, $df = 7$).

Whether foragers were exposed to leaf disks or leaf odors, acceptance decreased significantly between control series and treatment series in both set-ups and both tested plant pairs due to experience with the unsuitable substrate. Did foragers show the same magnitude of change in acceptance when deciding between leaf disks of two plant species than when they are exposed to plant odors only? To see if the magnitude of change in acceptance is dependent on the plant characteristics foragers are given to make a decision, results from both experimental series were compared (Figure 1.4). The acceptance of blackberry offered with plum decreased by 68.2 ± 6.1 %, when foragers could decide between leaf disks of the two plant species tested.

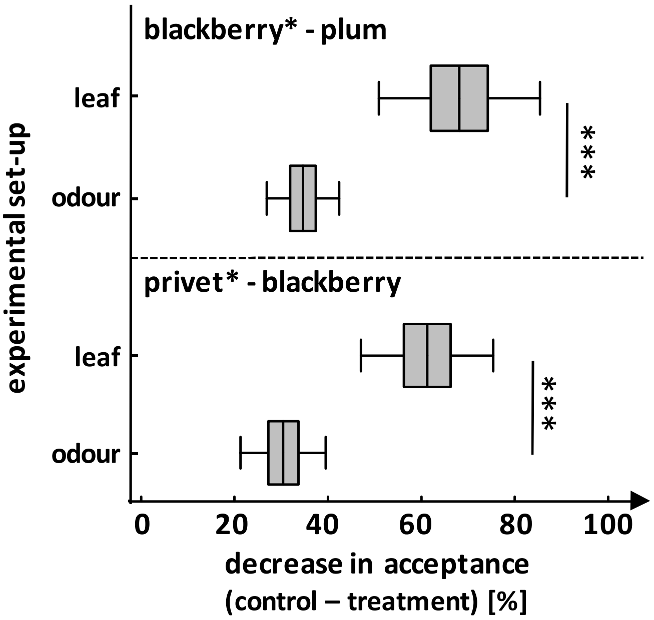

Figure 1.4: Comparison of decrease in acceptance of treated plant material between experimental set-ups. Decrease in acceptance was calculated as follows: difference in acceptance between control and treatment as percentage of control (= 100%). Data are mean ± SE, and n = 8 subcolonies for each group (with n = 40 individuals tested per subcolony). Significance of unpaired t-tests (leaf set-up - odor set-up) is given as ***p < 0.001.

In the odor set-up, foragers choosing blackberry odor decreased 34.7 ± 2.7 %. Decrease in acceptance of the formerly preferred substrate blackberry was only half as strong when foragers were offered plant odors instead of whole leaf disks (p < 0.001, t = -5.01, df = 14). The same pattern could be observed in the second plant pair tested, privet and plum (p < 0.001, t = -5.17, df = 14).

Discussion

Foragers are able to learn about substrates' suitability for their fungus inside the nest and acceptance decreases significantly within 24 hours after the initial harvest (Ridley et al. 1996; North et al. 1999; Herz et al. 2008; Saverschek et al. 2010) if the substrate proves to be unsuitable for the symbiotic fungus. As rejection occurs species specific (Herz et al. 2008; Saverschek et al. 2010) ants must be able to locally associate the negative effects of the substrate on their fungus with the incorporated substrate that causes it. Outside the nest, it has been shown before that odor can play an important role in the foraging process of leaf-cutting ants. During recruitment in *Acromyrmex lundi*, recruits are conditioned to the odor of the leaf fragment carried by the scout worker, and use the learned cue to decide which material should be collected at the food source (Roces 1990, 1994).

This study shows that foragers of *Acromyrmex ambiguus* recall experience they made with the plant species and its effects on the fungus inside the nest when encountering the plant odor outside. Individual foragers significantly preferred one of two suitable plant odors presented to them in a binary choice experiment on an established side trail. After subcolonies received treated leaf disks of the plant species which odor the foragers had preferred, significantly fewer foragers chose the odor of this plant species the following day. Therefore, odor of the unsuitable plant species is enough for foragers outside the nest to retrieve the memory about the plants unsuitability for the fungus.

Most of the existing examples in which ants or bees learn about an odor inside the colony and adjust their foraging behavior accordingly are in the appetitive context leading to an increase in intake of the substrate in question. Workers of *Camponotus mus* e.g. are able to associate an odor with sucrose they receive through trophallaxis inside the nest and use this memory as choice criteria during food search (Provecho & Josens 2009). Honeybees can also learn odors inside the hive, where odor is either associated with the nectar or clinging on the successfully returning foragers' body and use the information in following foraging flights (von Frisch 1967; Wenner et al. 1969; Farina et al. 2005; Grüter et al. 2006). In bumblebees, floral scent in the colony was enough to trigger learning shown by a change in foraging behavior (Molet et al. 2009). Appetitive conditioning of the workers takes place inside the nest or hive and learned information can be retrieved outside during foraging at a later point in time. Transfer of a learned negative association to a different context has been recently reported in honeybees (Carcaud et al. 2009). Here, individuals trained to associate an odor with an electric shock avoided the odor in a choice situation in a Y-maze. In the experiments presented here, leaf-cutting ants recall information about the unsuitability of a substrate gained inside the nest which leads to avoidance of the odor of said substrate during foraging the following day. Contrary to honeybees, leaf-cutting ants do not experience the negative effects themselves, but aversive olfactory conditioning takes place as workers are influenced through the effects of the substrate on their symbiotic fungus.

The ability of leaf-cutting ants to distinguish between two simultaneously presented plant odors leads to the conclusion that odor might also be enough to identify an incorporated substrate inside the nest. Nonetheless, as there should still be gustatory characteristics of the incorporated substrate available, leaf-cutting ants presumably use olfactory together with gustatory cues for the identification inside the nest. Molet et al. (2009) showed in bumblebees that learning was improved when odor was provided in nectar in the honey pots instead of only in the air of the hive, probably because gustatory cues were involved additionally and a direct association of odor and nectar was possible.

When foragers choose between leaf disks, they are exposed to more plant characteristics like secondary compounds as well as physical parameters that might influence their plant choice (Howard 1987, 1988; Cherrett 1989; Nichols-Orians & Schultz 1990; Saverschek et al. 2010). Significantly more foragers rejected the unsuitable plant species after the treatment when choosing between leaf disks of the two tested species instead of plant odors. It is not surprising that the presence of more parameters involved in identification and evaluation of the substrate leads to a more distinct decision. This result further strengthens the assumption that gustatory characteristics also play a role next to olfactory characteristics in the identification of plant material in the fungus.

In the foraging context, individuals exhibited avoidance of the direction of the odor of the unsuitable substrate, moving away from it. This locomotion response suggests plant recognition from a distance through odor and consequent avoidance of that direction also occurs out in the field. This could increase foraging efficiency as foragers avoid further harvesting of said substrate earlier, therefore decreasing subsequent post-selection inside the nest and individuals are able to engage in the harvest of other, more suitable substrates earlier.

CHAPTER 2

SOURCES OF INFORMATION ABOUT PLANT SUITABILITY INSIDE THE NEST:

FUNGUS, GARDENERS AND FORAGERS

Abstract

Foraging patterns of leaf-cutting ants are the result of an intricate interplay between the different members of the colony involved in the harvesting process: foragers, gardeners and the symbiotic fungus. So far it is known that foragers can gain information about substrate suitability throughout the foraging activity outside the nest, directly at the foraging site through evaluation of the substrate and through interactions with other foragers on the trail. Inside the nest, foragers can learn about the suitability of the harvested substrate through their symbiotic fungus. I could show that foragers without own foraging experience change their substrate preferences based solely on experiences gained in the fungus garden and determined several conditions necessary for learning about substrate suitability inside the nest. Foragers learn about the unsuitability of a substrate and its identity if it has been freshly incorporated and the effects on the fungus are still detectable. When the association between the condition of the fungus and the incorporated substrate cannot be made anymore, gardeners alone are enough to communicate unsuitability of the substrate. Their negative experience in the past lowers the acceptance of naïve foragers to now suitable substrate even though aversive learning is slightly stronger when the effect of the unsuitable substrate on the fungus is still detectable than when only the gardeners had experience with the substrate in the past. Aversive learning is stronger in the additional presence of experienced foragers indicating information transfer between foragers as well. Experienced foragers alone though are not enough to lower the acceptance of substrate by naïve foragers in the presence of naïve gardeners, even if experienced foragers make up the majority of the workforce. Experienced foragers are also able to reverse their previous experience about the unsuitability of a substrate and start accepting it again. However, aversive learning is stronger than appetitive learning. Various scenarios in which foragers are able to learn about substrate suitability inside the nest, either from their fungus or their nestmates show that leaf-cutting ants constantly evaluate, learn about and re-evaluate the suitability of harvested substrate and adjust their foraging behavior accordingly. The existence of several ways information about substrate suitability is distributed might facilitate speed as well as accuracy of substrate selection and therefore the foraging efficiency of the colony.

Introduction

Leaf-cutting ants are highly polyphagic herbivores due to their association with their symbiotic fungus. In this cooperation, the harvested plant material gets brought inside the fungus chambers where workers cut down the leaf fragments and gardeners incorporate the substrate. The fungus provides the ants with food for their developing brood and larvae.

Leaf-cutting ants live in habitats with highly diverse plant communities. They harvest 50 - 80 % of the occurring plant species, normally several plant species simultaneously (Cherrett 1989; Wirth et al. 2003). Out in the field, foragers decide on plant material for their symbiotic fungus based on innate tendencies and experience. A second level of quality control occurs inside the nest, in the fungus gardens. Physical plant characteristics like hardness evaluated by foragers throughout processing can lead to post-selection of plant material (Camargo et al. 2003) and, more importantly, if the substrate proves to be unsuitable for the fungus due to secondary compounds, foragers adjust their foraging behavior accordingly, i.e. stop harvesting said substrate (Ridley et al. 1996; North et al. 1999).

An adult colony normally consists of thousands of foragers that supply the fungus chambers with a variety of fresh plant material via several main foraging trails. The harvested substrates are usually distributed homogenously throughout the nest within 24 - 48 hours (Forti & Silveira Neto 1989; Pretto & Forti 2000; Moreira et al. 2003). If a substrate after its incorporation into the fungus proved itself unsuitable, rejection on the numerous foraging trails of the colony occurs at different time intervals after the initial harvest. Field studies with *Atta colombica* have shown that rejection of the substrate first occurred on the trail that originally harvested the unsuitable substrate (within 24 hours) and then on the adjacent trails (24 - 48 hours; Saverschek et al. 2010). This time delay leads to a temporary discrepancy of experiential backgrounds of foragers regarding harvested substrates, based on their whereabouts, meaning the trail they harvested on and the fungus gardens they visited at certain times. Experience seems to be one of the key factors explaining the variance in plant preferences of leaf-cutting ant colonies. As their environment is seasonally changing (Fowler & Stiles 1980) the flexibility of foraging preferences helps leaf-cutting ant colonies to respond.

In order to gain experience foragers can either gather information about substrate suitability in the field throughout the foraging process or in the fungus garden throughout the processing and incorporation of the harvested material. Many studies have looked at influences during the foraging cycle, but few have focused on information flow inside the nest.

The phenomenon of delayed rejection has already been shown in several leaf-cutting ant species (*Ac. lundi, Ac. octospinosus, Ac. subterraneus, A. cephalotes, A. laevigata, A. sexdens, A. colombica*) both in the laboratory (Knapp et al. 1990; Ridley et al. 1996; North et al. 1999;

Camargo et al. 2003; Herz et al. 2008) and in the field (Ridley et al. 1996; Wagner 2004; Saverschek et al. 2010). It is still unknown how the information passes from the fungus to the workers of the colony. The suggestion of a chemical mediator in the rejection process was first made by Ridley et al. (1996) after experiments with artificial bait proving the involvement of the symbiotic fungus in the process of the delayed rejection of a substrate. Experiments conducted with *Atta sexdens* in the lab have ruled out a highly volatile component and lead to the idea of a semiochemical that might be passed from the fungus via the gardening workers to the foragers without necessarily passing directly between the fungus and the foragers (North et al. 1999). The information in the fungus garden only seems to last for a few days (Herz et al. 2008), but experienced gardeners are still present in the fungus garden afterwards.

This study tried to unveil information flow about substrate suitability within the nest, from the fungus to the workers, among workers, between workers of different castes or with different experiential background. Several experimental series were conducted to elucidate under which conditions foragers without own foraging experience can learn about substrate suitability in the fungus garden. Specifically, I addressed the following questions: Can foragers of *Acromyrmex ambiguus* only learn about the suitability of a substrate if they have harvested, processed and incorporated it into the fungus themselves or can they also gain information about the suitability of substrates harvested by others? If the information about plant suitability is no longer retrievable from the fungus, can experienced gardeners influence the foraging decisions of naïve foragers? If so, under which conditions? Can experienced foragers influence the preference of naïve foragers through their presence in the nest? Can foragers learn about the suitability of a substrate equally well as about the unsuitability of a substrate inside the nest (aversive versus appetitive learning)?

Methods

I tested if and under which conditions foragers can learn about the suitability of a given substrate in the fungus garden without own foraging experience with this substrate. To these means, transfer experiments were conducted in which foragers that differed in their experiential background regarding the tested substrate were transferred into fungus gardens that also differed in their experience with the tested substrate. A fungus garden always consisted of the fungus with its gardening workers. Standardized preference tests were used to test the foragers' preference before and 24 h after the transfer.

Subcolonies

Experiments were conducted with subcolonies obtained from 5 large lab colonies of *Acromyrmex ambiguus*. The colonies were collected in Uruguay in 2002 and maintained in the laboratory at 25°C and under a LD cycle of 12:12 h. Artificial nests consisted of three transparent Plexiglas®-boxes connected by short PVC-tubes (10 cm, Ø 3 cm). The center box (19 x 8.5 x 8.5 cm) served as the fungus chamber and the two other boxes (19 x 19 x 8.5 cm) as feeding arena and refuse-box respectively. The bottom of the fungus-box was covered with moistened expanded clay pebbles to keep the humidity high and prevent desiccation of the fungus. The lids of the refuse- and feeding-boxes had three holes each (Ø 3 cm), covered with a fine metallic mesh allowing air circulation to create a different, dryer microclimate from the one inside the fungus-box, an *inside* and *outside* for the subcolony.

About 1000 ml of fungus garden (fungus and gardening workers) were taken from the mother colony and placed in the artificial nest together with approximately 1000 foragers. Subcolonies were established at least 4 days prior to the start of the experiment to ensure well established fungus gardens and active foraging behavior. The subcolonies received fresh blackberry leaves (*Rubus fructicosus*) and water every day and honey water every other day.

Experimental set-up

The subcolony was connected to a wooden ramp that led out of the standard feeding box onto a 5 cm wide and 4 m long wooden bridge (*main trail*) a wooden platform (10 x 10 cm) were the preference tests were conducted (see Figure 2.1). Subcolonies foraged along the main trail for at least 2 days before the start of the experiment.

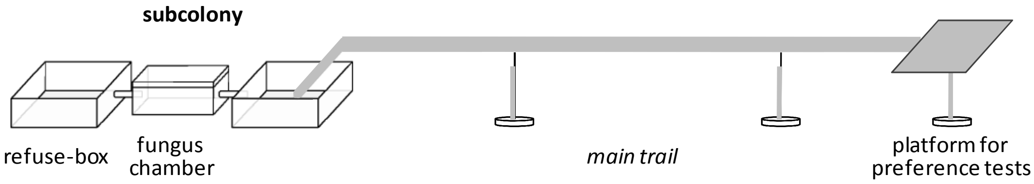

Figure 2.1: Experimental set-up. A ramp led foragers on a bridge which connected the subcolony to the platform for preference tests 4 m away.

Experimental procedure

Standardized preference tests were used three times throughout the course of the experiment to measure the acceptance of privet by foragers. The procedure was always the same. The subcolony was given time (between 20-30 min) to establish the foraging process along the main foraging trail, until a constant number of laden foragers returned from the main patch (consisting of blackberry leaves) to the colony. 20 leaf disks (Ø 6 mm) of each plant, privet and blackberry, were then offered simultaneously on the wooden platform at the end of the main trail. Whenever a leaf disk was picked up and carried off the platform, it was immediately replaced by a leaf disk of the same type (*ad libitum*). Foragers leaving the platform with their load were recorded. The test was conducted for 25 min.

Treatment of subcolonies: to create different experiential backgrounds, subcolonies were exposed to one of two treatments. They either received 110 untreated, suitable leaf disks of privet (from now on: *naïve* subcolonies) or 110 leaf disks of treated, unsuitable privet (from now on: *experienced* subcolonies). The treated leaf disks were infiltrated with CHX (see chapter 1). The privet leaf disks were offered simultaneously together with untreated blackberry leaf disks in form of a standardized preference test (no. 1). The test was recorded in 58 randomly selected subcolonies and served as a control to verify that both offered plant species were accepted equally well before the treatment.

Transfers of foragers to a different fungus garden took place on day 1 or day 7 after the treatment (Figure 2.2). Before the transfer, a preference test (no. 2) was conducted to establish the *status quo* of the subcolonies after the treatment. Studies with *Acromyrmex lundi* have shown that naive foragers were only able to retrieve the information about the unsuitability of a substrate if they were transferred within 2 days after the incorporation of the substrate (Herz et al. 2008). Day 1 and 7 after the treatment were chosen to have *experienced* fungus gardens with and without the effects of the unsuitable substrate on the fungus still detectable.

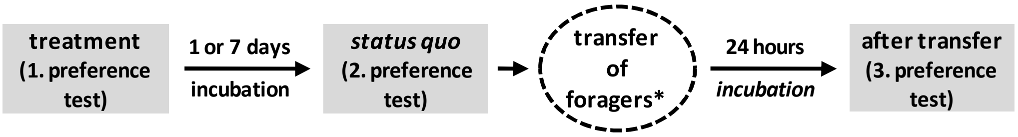

Figure 2.2: Time line of general experimental procedure for all series. *with / without 50 leaf disks (depending on the series).

All foragers were then collected from the subcolony, so that only the fungus with its gardening workers remained (*fungus garden*). In order to retrieve the foragers from the artificial nest, the fungus-box was separated from the refuse- and feeding-box and all workers outside the fungus-box were collected. Foragers tend to come out of the garden when the nest gets disturbed. The lid of the fungus-box was also lifted and large individuals were carefully collected of the top of the fungus with featherweight forceps. The whole procedure did not exceed 45 min and the fungus-box was kept shut as much as possible to minimize desiccation of the fungus. Once all foragers were collected from the fungus, the fungus-box containing the fungus and its gardeners was connected again with the empty feeding- and refuse-box. Now 700 foragers were added to the refuse-box and the artificial nest was closed again. After a 24 h *incubation time*, the foragers' preference was then tested again (no.3). Foragers were added to the refuse box to make sure that any forager that appeared on the main trail during the next preference test had passed the fungus garden.

Seven experimental series and one control series were conducted (Table 2.1). Depending on the series, transferred foragers were only *naïve* (A, B & E), only *experienced* (G & control) or a combination of both (C, D & F). If *naïve* and *experienced* foragers were transferred together, the ratio was always 200 *naïve* foragers versus a majority of 500 *experienced* foragers. Four series (D-G) additionally received 50 untreated leaf disks of the previously unsuitable substrate (privet) that were also directly placed on top of the fungus garden inside the fungus-box.

Table 2.1: Overview of experimental series conducted. Tested combinations of foragers and fungus gardens with different experiential background.

experimental series	time of transfer	foragers	fungus garden	leaf disks added	n
A	**day 1**	only naïve	experienced		10
B	**day 7**	only naïve	experienced		7
C	**day 7**	naïve & experienced	experienced		7
D	**day 7**	naïve & experienced	experienced	X	9
E	**day 7**	only naïve	experienced	X	7
F	**day 7**	naïve & experienced	naïve	X	10
G	**day 7**	only experienced	naïve	X	8
control	**day 7**	only experienced	experienced		7

How learning of *naive* foragers about the unsuitability of a substrate might be influenced by the elapsed time since the incorporation of unsuitable substrate was investigated in series A and B. I then looked at the influence of presence or absence of *experienced* foragers and fresh suitable substrate in *experienced* fungus gardens on preferences of *naive* foragers (series C, D & E) and on the influence of *experienced* foragers on *naive* foragers in a *naive* fungus garden (Series F). To see if there is a difference between aversive and appetitive learning, *experienced* foragers were transferred to *naive* fungus gardens in series G. The control series was conducted to account for possible effects of the transfer itself.

If a mixture of *naive* and *experienced* foragers was added to a subcolony, one group of ***foragers was marked*** with a dot of paint (Edding® 750) on the thorax. The paint was carefully applied with the tip of a bent paper clip. Marked workers were left in a separate box for several minutes to dry before they were put back in the colony. No deleterious effect of the paint could be observed.

Data analysis

Observer 2.0®, a DOS-based program for observational data collection (Noldus Information Technology, Wageningen, Netherlands) was used to record the observations from the preference tests.

The ***standardized acceptance of privet*** was calculated by dividing the number of privet leaf disks picked up and taken away through the number of all leaf disks picked up and taken throughout the preference test (25 min). Values for standardized acceptance therefore ranged from 0.0 to 1.0 with a value of 0.5 indicating equal acceptance of both offered plant species. Acceptance of privet *before transfer* and *after transfer* was compared in all experimental series.

To comply with requirements of normality, all values were arcsine transformed prior to statistical analyses. Status quo of *naïve* and *experienced* subcolonies before the start of the transfer experiments was established. Significance of difference in acceptance of privet of *naïve* subcolonies from random distribution *before transfer* was tested with t-test for single mean. Standardized acceptance of privet of *experienced* subcolonies *before transfer* was tested comparing acceptance *before treatment* and *after treatment* with unpaired t-tests.

Change in acceptance between *before transfer* and *after transfer* was calculated as follows and compared across experimental series. 100 % was defined as the acceptance of privet by *naïve* subcolonies minus the acceptance of privet by their paired *experienced* subcolonies *before transfer*. Acceptance of privet *after transfer* was calculated as percentage of these.

A square root transformation was used prior to statistical analyses to comply with requirements of normality and differences in changes of acceptance between different experimental series were tested with unpaired t-tests.

Results

Before the start of the experimental series, the experiential background of *naïve* and *experienced* subcolonies in regards to the acceptance of privet was verified.

The *status quo* of subcolonies that had received untreated privet leaf disks (n = 48; preference test no.1) was calculated based on the results of the preference test conducted before the transfer (no.2). Privet and blackberry were equally suitable plant species with a standardized acceptance of privet of 0.49 ± 0.09 ($p = 0.53$, $t = -0.63$, $df = 57$; t-test for single mean). The subcolonies were therefore considered *naive* regarding possible negative effects of privet on their fungus.

The acceptance of privet by subcolonies that had received fungicidal leaf disks was tested separately for each experimental series (Table 2.2). All treated subcolonies showed a significantly lower acceptance of privet after the treatment with fungicidal leaf disks (preference test no.2) compared with the acceptance of privet before the treatment (no.1). Treated subcolonies, their foragers and fungus gardens containing gardening workers were therefore considered *experienced* regarding possible negative effects of privet on their fungus.

Table 2.2: Overview of the acceptance of privet of *experienced* subcolonies and their use in the experimental series. Differences in standardized acceptance of privet (mean ± SE) between *before* and *after treatment* with fungicidal leaf disks was tested with paired t-tests.

experimental series (n)		**components of subcolonies used**	**stand. acceptance of privet**		
			before treatment	**after treatment**	
A	(10)	fungus garden	0.41 ± 0.08	0.07 ± 0.05	$p \leq 0.001$
B	(7)	fungus garden	0.56 ± 0.06	0.14 ± 0.07	$p \leq 0.001$
C	(7)	fungus garden & foragers	0.53 ± 0.07	0.08 ± 0.09	$p \leq 0.001$
D	(9)	fungus garden & foragers	0.46 ± 0.07	0.04 ± 0.04	$p \leq 0.001$
E	(7)	fungus garden	0.56 ± 0.09	0.11 ± 0.08	$p \leq 0.001$
F	(10)	foragers	0.58 ± 0.08	0.08 ± 0.05	$p \leq 0.001$
G	(8)	foragers	0.51 ± 0.03	0.10 ± 0.03	$p \leq 0.001$
control	(7)	fungus garden & foragers	0.49 ± 0.07	0.07 ± 0.06	$p \leq 0.001$

The control series was conducted to account for potential effects of the transfer itself. 700 experienced foragers were transferred into a fungus garden 7 days after the incorporation of fungicidal privet ($n = 7$). No differences in the foraging behavior of the transferred foragers before and after the transfer were observed. In both preference tests, before (0.06 ± 0.06) and after the transfer (0.10 ± 0.09), foragers hardly accepted the previously unsuitable privet and foraged mainly on the simultaneously offered blackberry leaves, proving that transfer itself had no influence on the foraging behavior ($p = 0.49$, $t = -0.74$, $df = 6$; paired t-test).

Influence of elapsed time since incorporation of unsuitable substrate

I tested if *naive* foragers can learn about the unsuitability of a substrate after being exposed to an *experienced* fungus garden for 24 hours (Figure 2.3). Two series were conducted. In series A, *naive* foragers were transferred to a fungus garden 1 day after the incorporation of unsuitable privet, in series B the transfer took place 7 days after the incorporation.

Naïve foragers that were transferred one day after the incorporation of unsuitable substrate had significantly lowered their acceptance of this substrate after 24 hours. Foraging experience was not necessary to learn about substrate quality, as *naive* foragers learned about the unsuitability of a substrate through exposure to the fungus garden alone (series A: $p < 0.001$, $t = 7.22$, $df = 9$). If *naive* foragers were transferred into a fungus garden seven days after its treatment, they did not change their acceptance of the substrate after the 24 hours *incubation time*, which shows that either there was no more information about the substrate's suitability present in the fungus garden, or they could not retrieve the information about the plant's identity anymore (series B: $p = 0.20$, $t = 1.42$, $df = 6$).

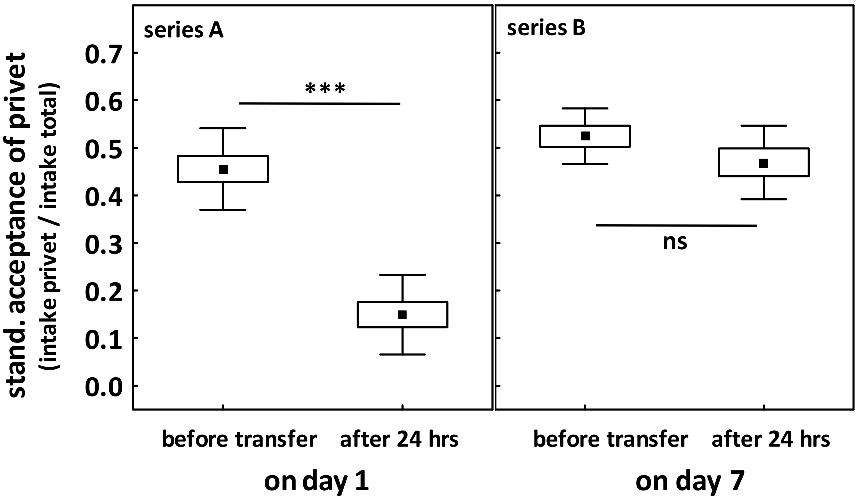

Figure 2.3: Acceptance of privet by *naive* foragers transferred to an *experienced* fungus garden 1 day / 7 days after the treatment (series A / B). 700 *naive* foragers were transferred and acceptance of privet was tested before and after 24 hours. Data are mean ± SE, with day 1: n = 10 and day 7: n = 7 subcolonies. Significance of paired t-tests is given as ns = non significant and ***p < 0.001.

Experienced fungus gardens had a significant influence on the acceptance of privet by *naive* foragers one day after the treatment, but not seven days later.

Influence of presence or absence of experienced foragers and fresh suitable substrate

To investigate if the presence of a majority of *experienced* foragers could influence the *naïve* foragers' acceptance of privet, we conducted another experimental series (series C) in which 200 *naïve* foragers and 500 *experienced* foragers were transferred to a 7 day old fungus garden (Figure 2.4). *Naïve* and *experienced* foragers showed no changes in their acceptance of the tested substrate (*naïve*: p = 0.73, t = -0.36, df = 6; *experienced*: p = 0.74, t = -0.35, df = 6). *Naive* foragers still accepted the substrate well, whereas *experienced* foragers still rejected the substrate after the 24 hours *incubation time*. Therefore, the presence of a majority of *experienced* foragers in the fungus garden did not lead to a decrease in acceptance of privet by *naïve* foragers.

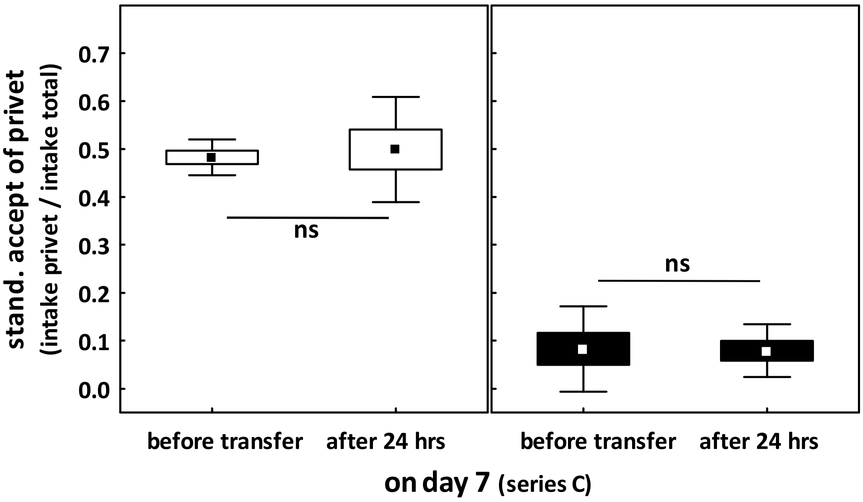

Figure 2.4: Acceptance of privet by *naive* and *experienced* foragers transferred to an *experienced* fungus garden 7 days after the treatment (series C). 200 *naive* (☐) and 500 *experienced* (■) foragers were transferred and acceptance of privet was tested before and after 24 hours. Data are mean ± SE with n = 7 subcolonies. Significance of paired t-tests is given as ns = non significant.

To investigate if the lack of identifiable substrate might be the reason for the lack of communication between *experienced* and *naïve* workers I conducted experimental series D (Figure 2.5). A mixture of *naïve* and *experienced* foragers was transferred to a fungus garden 7 days after the treatment and 50 leaf disks of untreated privet were also added directly to the fungus garden. After the 24 h *incubation time*, the 50 added leaf disks seemed to have been incorporated into the fungus as they could not be seen with the naked eye anymore and did not show up on the refuse pile. If *naïve* foragers or *experienced* foragers or gardeners were responsible for the incorporation of the substrate could not be established. Even though the added leaf disks were untreated, *naïve* foragers now showed a significant decrease in acceptance of the substrate after 24 hours (p < 0.001, t = 7.79, df = 8). In this case, *naïve* foragers lowered their acceptance of privet without experiencing the negative effects on the fungus themselves. Their rejection could only be based on information received from *experienced* nestmates, either foragers or gardeners or both. It is also interesting to see that even though the substrate added to the fungus garden was suitable and *naive* foragers were also present, the low acceptance of privet by *experienced* foragers did not change (p = 0.65, t = 0.47, df = 8).

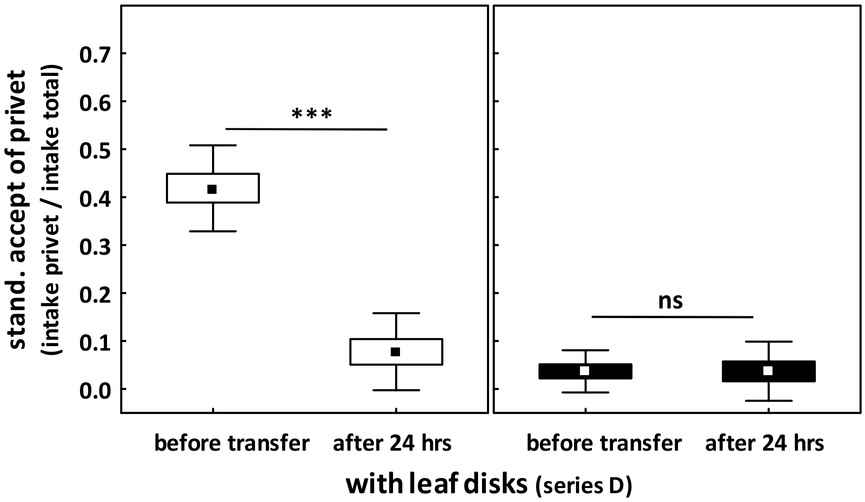

Figure 2.5: Acceptance of privet by *naive* and *experienced* foragers transferred with untreated leaf disks to a fungus garden 7 days after the treatment (series D). 200 *naive* (☐) and 500 *experienced* (■) foragers were transferred with 50 untreated leaf disks and acceptance of privet was tested before and after 24 hours. Data are mean ± SE with n = 9 subcolonies. Significance of paired t-tests is given as ns = non significant and ***p < 0.001.

Experimental series E was conducted to test if the presence of a majority of *experienced* foragers in the fungus garden is necessary for *naïve* foragers to learn about substrate quality or if the presence of *experienced* gardeners is enough if the substrate is present. Only *naïve* foragers together with 50 untreated leaf disks of privet were transferred into a fungus garden that had been treated seven days previously. In this series, the *experienced* gardeners were the only source of information about the former unsuitability of the present substrate. Figure 2.6 shows that *naïve* foragers significantly lowered their acceptance of privet after the 24 hours *incubation time* (p < 0.001, t = 5.37, df = 6). The presence of *experienced* gardeners was enough to change the acceptance of privet by *naïve* foragers even though the substrate present was suitable.

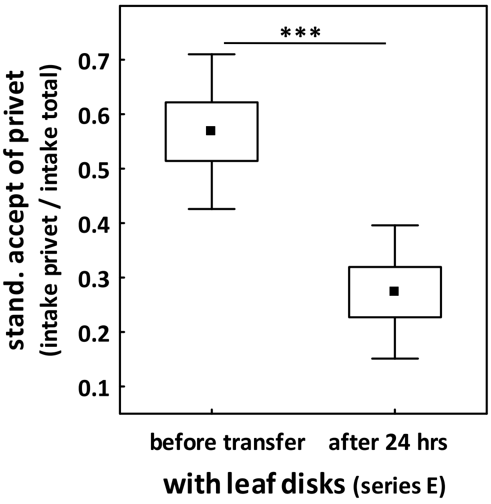

Figure 2.6: Acceptance of privet by *naive* foragers transferred with untreated leaf disks to a fungus garden 7 days after the treatment (series E). 700 *naive* foragers were transferred with 50 untreated leaf disks and acceptance of privet was tested before and after 24 hours. Data are mean ± SE with n = 7 subcolonies. Significance of paired t-test is given as ***p < 0.001.

The presence of experienced foragers is not necessary for *naive* foragers to learn about previous unsuitability of privet.

The question that arises now is, if *naïve* foragers learn at all about the substrate quality from *experienced* foragers or if they only learn through the gardeners. Too see if the presence of *experienced* foragers had a significant influence on the decrease of acceptance of privet by *naive* foragers, I compared the change in acceptance by *naive* foragers with (series D) and without (series E) *experienced* foragers present (Figure 2.7).

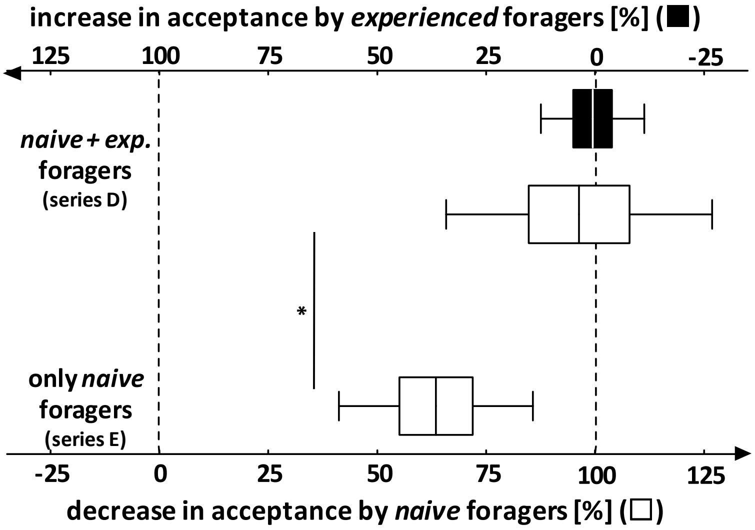

Figure 2.7: *Naive* foragers in *experienced* fungus garden with 50 untreated leaf disks 7 days after the treatment. Change of acceptance after 24 hours with (series D) and without *experienced* foragers present (series E). Data are mean ± SE, with n = 9 (series D) and n = 7 (series E). 100 % = Differences in acceptance between *naïve* and *experienced* subcolonies *before the transfer*. Series D: *naive before*: 0.40 ± 0.07, *exp. before*: 0.04 ± 0.05, 100 % = 0.35 ± 0.06. Series E: *naive before*: 0.57 ± 0.14, *exp. before*: 0.11 ± 0.08, 100 % = 0.46 ± 0.17. Significance of unpaired t-test is given as *p < 0.05.

The top x-axis shows the increase in acceptance by *experienced* foragers and the lower x-axis shows the decrease in acceptance of privet by *naive* foragers. In series D, *naive* foragers were transferred together with a majority of *experienced* foragers and their acceptance of privet decreased by about 95 %. If *naive* foragers were transferred alone, without *experienced* foragers (series E), their acceptance of privet decreased about 65 % after the 24 h *incubation time*. The presence of a majority of *experienced* foragers therefore had a significant influence on the decrease of acceptance of privet by *naive* foragers (p = 0.04, t = -2.29, df = 12). *Naïve* foragers were not only influenced by *experienced* gardeners, but also by *experienced* foragers in their acceptance of privet.

Naive foragers decrease their acceptance of a substrate even if the substrate is suitable if the gardeners had a negative experience with it seven days previously (Figure 2.6, series E). In this situation, foragers only gained information through *experienced* gardeners if substrate was present.

Influence of presence or absence of information in the fungus

Transferring *naive* foragers on day 1 after the treatment, they are exposed to a fungus garden containing *experienced* gardeners and the effect of the unsuitable substrate on the fungus (Figure 2.3, series A). It is unclear if foragers are able to obtain information about the suitability of a substrate from the fungus itself, or if they only gain that information though the gardeners that tend the fungus.

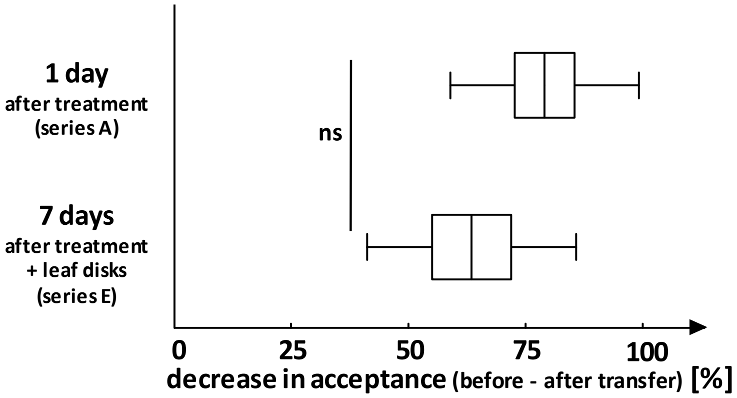

Figure 2.8: Naive foragers in experienced fungus garden 1 and 7 days after the treatment. Change of acceptance after 24 hours with (series A) and without the effects on the fungus present (series E). Data are mean ± SE, with n = 10 (series A) and n = 7 (series E). 100 % = Differences in acceptance between naïve and experienced subcolonies before the transfer. Series D: naive before: 0.46 ± 0.09, exp. before: 0.07 ± 0.06, 100 % = 0.39 ± 0.09. Series E: naive before: 0.57 ± 0.14, exp. before: 0.11 ± 0.08, 100 % = 0.46 ± 0.17. Significance of unpaired t-test is given as ns = non significant.

I therefore compared the decrease in acceptance of privet by *naive* foragers based on the presence (series A) or absence (series E) of the effects on the fungus (Figure 2.8). The change in acceptance of the previously treated plant species by *naïve* foragers was slightly higher on day 1 than on day 7, but not significantly ($p = 0.15$, $t = 1.50$, $df = 15$). *Naïve* foragers could learn just as well through *experienced* gardeners on day 7 without the effects of the substrate present in the fungus as they learned on day 1 with the effects on the fungus present. It is therefore still unclear if *naïve* foragers can only gain information through the gardeners or if they can also gain the information directly from the fungus.

Influence of experienced foragers in naïve fungus garden

The presence of *experienced* gardeners is enough to decrease *naïve* foragers' acceptance of privet, even if the substrate is suitable (series E). The additional presence of *experienced* foragers significantly decreases *naïve* foragers' acceptance of privet even further (Figure 2.7). The question arising from these results is, if the presence of a majority of *experienced* foragers in a *naïve* fungus garden alone is enough to lower the acceptance of privet of *naïve* foragers. *Experienced* and *naïve* foragers were transferred to a *naïve* fungus garden on day 7 together with 50 leaf disks of suitable privet (series F).

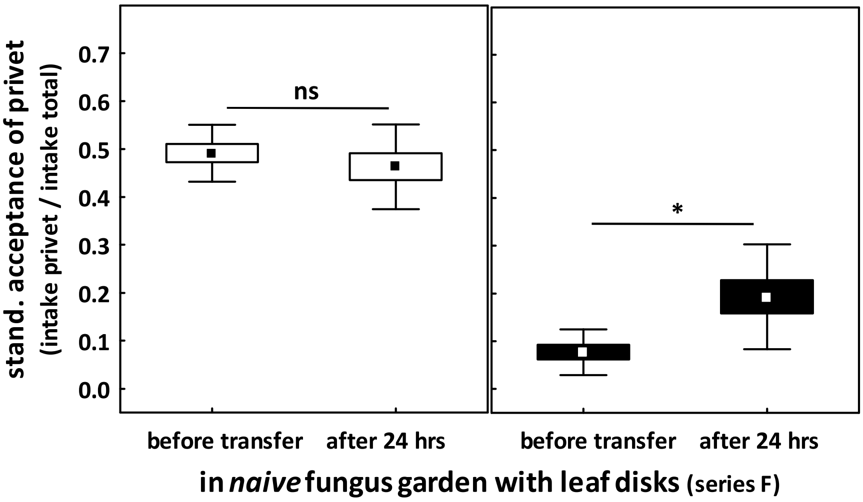

Figure 2.9: Acceptance of privet by *naive* and *experienced* foragers transferred with untreated leaf disks to a *naive* fungus garden 7 days after the treatment (series F). 200 *naive* (☐) and 500 *experienced* (■) foragers were transferred with 50 untreated leaf disks and acceptance of privet was tested before and after 24 hours. Data are mean ± SE with n = 10 subcolonies. Significance of paired t-tests is given as ns = non significant and *p < 0.05.

The presence of a majority *experienced* foragers alone had no effect on the acceptance of privet by *naïve* foragers in a *naïve* fungus garden. They still accepted privet 24 hours after the transfer ($p = 0.42$, $t = 0.85$, $df = 9$). Surprisingly though, acceptance of privet by *experienced* foragers had increased significantly ($p < 0.05$, $t = -2.76$, $df = 9$). In series D, *experienced* foragers still recalled their negative experience with a substrate after 7 days and kept rejecting it even if the substrate in the fungus garden was suitable and *naïve* foragers were present as well. With the additional presence of *naive* gardeners though, *experienced* foragers started accepting the formerly unsuitable privet again, showing that negative experience with a substrate can be reversed.

Aversive versus appetitive learning

The question is, if *naïve* gardeners can influence the preference of *experienced* foragers. In experimental series F, only *experienced* foragers were transferred together with 50 untreated leaf disks into a *naïve* fungus garden on day 7, to see if the presence of *naïve* gardeners together with now suitable privet would lead to an increase in acceptance of privet by the *experienced* foragers (Figure 2.10).

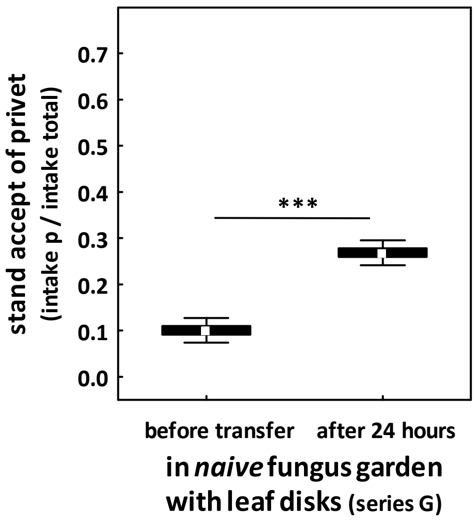

Figure 2.10: Acceptance of privet by *experienced* foragers transferred with untreated leaf disks to a *naive* fungus garden 7 days after the treatment (series G). 700 *experienced* foragers were transferred with 50 untreated leaf disks to *naive* fungus gardens and acceptance of privet was tested before and after 24 hours. Data are mean ± SE with n = 8 subcolonies. Significance of paired t-test is given as ***p < 0.001.

After 24 hours of *incubation time*, *experienced* foragers had increased their acceptance of privet significantly showing that foragers are able to alter their preference again and adapt to the new quality of an already known substrate ($p < 0.001$, $t = -10.47$, $df = 7$). They are able to change an already formed preference when exposed to a change in substrate suitability in the presence of *naïve* gardeners.

To see if foragers could relearn that a substrate is suitable again (series F) as well as they learned about the unsuitability (series E) in the beginning, the change of acceptance of the substrate was compared between the two series (Figure 2.11). *Naive* foragers learned significantly better about the unsuitability of the substrate than *experienced* foragers about the suitability of the substrate ($p = 0.012$, $t = 2.89$, $df = 12$). This is even more surprising as *experienced* foragers had suitable substrate and *naive* gardeners as potential sources of information in series F, whereas in series E, *naive* foragers were exposed to a fungus with *experienced* gardeners on the one hand, but suitable substrate on the other.

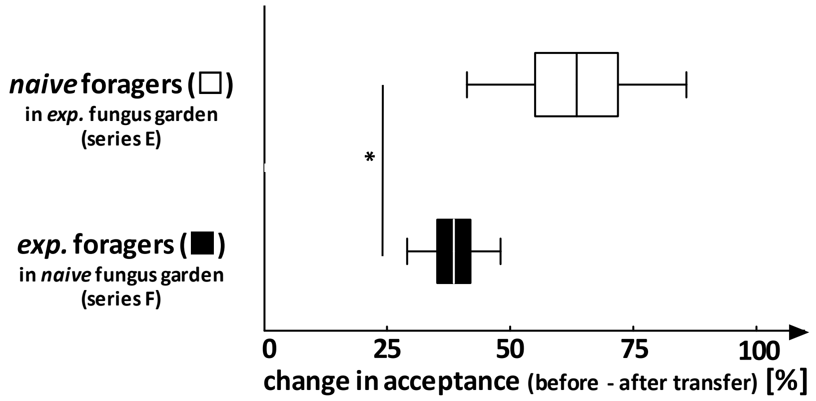

Figure 2.11: Change in acceptance of privet by *naive* and *experienced* foragers 24 hours after the transfer in a fungus garden with the opposite background. Change of acceptance after 24 hours with and without experienced foragers present. Data are mean ± SE, with n = 7 (series E) and n = 8 (series F). 100 % = Differences in acceptance between *naïve* and *experienced* subcolonies *before the transfer*. Series E: *naive before*: 0.57 ± 0.14, *exp. before*: 0.11 ± 0.08, 100 % = 0.46 ± 0.17. Series F: *naive before*: 0.54 ± 0.03, *exp. before*: 0.10 ± 0.03, 100 % = 0.44 ± 0.02. Significance of unpaired t-test is given as $^*p < 0.05$.

Discussion

The capability of learning about plant suitability seems to be one of the key factors explaining how leaf-cutting ant colonies can change their plant preferences and adapt their foraging patterns to their highly diverse and seasonally changing environment. The fact that leaf-cutting ants are influenced by the response of their symbiotic fungus in their foraging decisions adds a layer of complexity. It is of great interest to understand the information flow under different conditions which enables workers to gain information about substrate suitability in the fungus garden.

It has already been shown in several studies with *Atta* and *Acromyrmex* that foragers are able to learn about the unsuitability of a substrate through the reaction of their symbiotic fungus (Ridley et al. 1996; North et al. 1999; Herz et al. 2008). Exchange experiments with small subcolonies of *Acromyrmex lundi* showed that foragers only lowered their acceptance of the previously unsuitable substrate if they were exposed to an *experienced* fungus garden within 24-48 hours after the treatment with unsuitable substrate. Foragers transferred to a fungus garden three days after treatment showed no change in acceptance anymore. It was concluded that either the changes in the fungus garden occur only for a brief period or the leaf characteristics used by the ants for plant recognition are no longer detectable and therefore the association between detrimental effects and plant species can no longer be formed (Herz et al. 2008).

In this study, foragers of *A. ambiguus* were transferred to fungus gardens one and seven days after the incorporation of substrate treated with fungicide to create situations in which (a) the information about substrate suitability was still detectable in the fungus and (b) information about substrate suitability was no longer retrievable from the fungus, because the association between plant species and effects on the fungus was no longer possible.

Our results were in accordance with Herz' findings in *A. lundi*. *Naïve* foragers transferred to a fungus garden one day after the incorporation of unsuitable substrate significantly lowered their acceptance of the substrate after a 24 hour exposure to the treated fungus garden. They had neither harvested nor processed or incorporated the unsuitable substrate into the fungus and changed their foraging behavior solely based on experience they had gained in the fungus garden. *Naïve* foragers transferred seven days after the treatment on the other hand still accepted the substrate in a preference test 24 hours later. These results further support the assumption that either the changes in the fungus or the identifiers of the plant that induced these changes are only detectable for a brief time period.

Adding untreated leaf disks of the plant that had induced these changes in the fungus seven days previously lead to a rejection of the substrate by *naïve* foragers 24 hours later. Several studies have shown that even though leaf-cutting ant colonies normally harvest several plant species simultaneously (Cherrett 1989; Wirth et al. 2003), rejection of a substrate is always species specific (Knapp et al. 1990; Herz et al. 2008; Saverschek et al. 2010). The incorporation of plant substrate in the fungus takes place on a very small scale and suitable substrate might be incorporated right next to substrate that proves to be unsuitable (Weber 1972; Herz et al. 2008; per. observation). Workers are able to make precise distinctions between the different substrates and their effect on the fungus.

Freshly harvested suitable plant material is not associated with fungus areas already harmed prior to the intake of the plant material. In this case, naïve foragers could only have gained the information from the *experienced* gardeners as the plant material itself was suitable. Foragers lowered their acceptance of the plant without having experienced any negative consequences for the fungus themselves.

Gardeners seem to play an important role in passing information about substrate suitability to the foragers. This is only possible in the presence of the substrate in question. It is surprising that *naïve* foragers encounter suitable substrate in the fungus garden and reject the substrate in preference tests based on the negative experience of other members of their colony - the gardening workers. There are two possible explanations for this finding. *Naïve* foragers encounter two opposing sources of information in the fungus garden, the substrate, which is suitable, and the *experienced* gardeners, which communicate unsuitability of said substrate because of their previous negative encounter with it.

The 'negative' information conveyed by *experienced* gardeners to *naïve* foragers is either more important than the actual suitability of the substrate, or foragers are not capable of identifying changes in the fungus caused by incorporated substrate. This would mean that there is a chain of information from the fungus via the gardening workers to the foragers. As changes in the fungus and the identity of the incorporated substrate are most likely perceived by workers through olfactory and gustatory cues, it is conceivable that gardeners might have a lower threshold than foragers regarding the detection of these cues. This would be contrary to previous findings of perceptual differences of odors in relation to body size in other contexts. In *Bombus terrestris*, larger individuals showed a higher odor-sensitivity than smaller individuals in electro-antennogram responses to given odor concentrations (Spaethe et al. 2007). The same relation was found in trail-following behavior of *Atta vollenweideri* and *Atta sexdens* where sensitivity increased with body size (Kleineidam et al. 2007). It therefore seems most likely that the 'negative' information from the gardeners has a higher impact than the positive information obtained from the incorporated leaf disks directly as it is more important to avoid unsuitable plants harmful to the fungus than to distinguish between different suitable plants. Even when the substrate is suitable again and taken into the nest by *naïve* foragers, rejection initially gets reinforced if gardening workers are *experienced*. Once the suitability has been proven through the incorporation of the substrate, the intake increases again (personal observation).

Transferring a majority of *experienced* foragers with a minority of *naïve* foragers into a *naïve* fungus garden with suitable leaf disks showed a different picture. After the significant influence of *experienced* gardeners on the preference pattern of *naïve* foragers discussed above it was surprising to see that a majority of *experienced* foragers in the presence of *naïve* gardeners did not have the same effect on the acceptance of previously unsuitable substrate by *naïve* foragers. *Naïve* foragers still accepted the substrate and *experienced* foragers significantly increased their acceptance of the substrate previously encountered as unsuitable again. Whether this was based on the presence of *naïve* gardeners specifically or just on the fact that the *naïve* gardeners together with the *naïve* foragers made up the majority of workers in the fungus remains elusive. That the majority's preference overrules the preference of the minority of workers, in this case the one of the *experienced* foragers, seems more likely though than the exceptionally strong influence of a specific caste, the gardeners.

Previous studies have speculated about lifetime memory regarding substrate quality and the possibility of a turnover of the foragers in a colony as an explanation for the resumption of harvesting a previously rejected substrate (Knapp et al. 1990; Ridley et al. 1996). I could show that *experienced* foragers of *Acromyrmex ambiguus* were able to relearn about the suitability of a substrate if they were transferred to a *naïve* fungus garden with suitable leaf disks of the previously unsuitable substrate. Interestingly, the change in acceptance of *experienced* foragers learning about the suitability of the previously unsuitable substrate was significantly lower compared with *naïve* foragers learning about the unsuitability of a previously suitable substrate. This could further support the theory that aversive learning might evolutionary be more robust than appetitive learning.

From outside the nest, it is already known that foragers influence each other in their foraging decisions on the trail throughout the foraging process (Howard et al. 1996; Roces 1990, 1994). Inside the nest, *experienced* foragers only had a significant influence on the *naïve* foragers' preferences in the presence of *experienced* gardeners. If the gardeners were *naïve*, the *experienced* foragers' opinion on substrate suitability had no impact on the preference of *naïve* foragers.

As mature colonies have several foraging trails leading to a number of underground fungus chambers, the colony is made up of foragers and gardeners with different experiences regarding the same substrates (see next chapter for possible mechanism). These gained experiences can be shared with other workers and foragers can learn to reject certain substrates based on the gardeners' 'negative' experiences with these substrates.

This diverse experiential background of the individuals and the transfer of information make it possible that the colony as a whole can react more flexible to changing substrate suitability than changes in the experiential background of the colony based solely on the turnover of the worker population would allow.

CHAPTER 3

INSIDE THE NEST: INDIVIDUAL BEHAVIOR OF GARDENERS AND FORAGERS IN THE PRESENCE OF SUITABLE AND UNSUITABLE PLANT MATERIAL

Abstract

It has long been known that host plant selection in leaf-cutting ants is a complex process. Due to surrounding vegetation, season and experience, plant preferences differ between colonies and fluctuate throughout colonies lifetimes. The symbiotic fungus plays a major role in shaping the foraging pattern of a colony through its feedback to foragers about plant suitability. This study describes behavior of gardeners and foragers inside the fungus chamber after the processing of plant material in order to shed some light on the mechanisms involved in the flow of information about substrate suitability from the fungus to the ants. Gardeners and foragers were individually marked and presented with small patches of processed plant material, suitable as well as unsuitable. Plant patches are treated by significantly more ants for a longer time if the substrate is unsuitable for the fungus. Interestingly, foragers are overall less active in the presence of unsuitable substrate than gardeners, contrary to the presence of suitable substrate where there is no difference between gardeners and foragers in their general activity as well as in respect to the handling of the plant patches. Besides the differing behavior towards the plant patches due to their suitability, gardeners as well as foragers also show significantly different contacting behavior in the two experimental series. If the plant is unsuitable for their fungus, significantly more gardeners as well as foragers initiate contacts with nestmates. These contacts also last significantly longer in the presence of unsuitable substrate. Based on these results, the influence of gardeners' experience with unsuitable substrate was investigated at a later point in time to have a closer look at the possible influence of gardeners on foragers. Experienced gardeners were combined with naïve foragers and substrate that was now suitable seven days after the gardeners' experience with its unsuitability and their behavior was recorded. If gardeners have negative experience with a now suitable substrate, significantly more antennate the plant patches than without negative experience, but the percentage of gardeners handling patches stays the same. There is no influence on the behavior of *naïve* foragers towards the plant patches, yet the *naïve* foragers' contacts with nestmates are significantly influenced by the presence of experienced gardeners. If the substrate is suitable and experienced gardeners are present, significantly more *naïve* foragers initiate contacts with other nestmates and these contacts last significantly longer than in the presence of *naïve* gardeners. This suggests that foragers are able to gain information about plant suitability not only through experienced gardeners but also from the fungus directly which makes it more likely for the individual to learn about substrate suitability sooner. In large leaf-cutting ant colonies with several fungus chambers, where different plant species are harvested simultaneously from different locations, effective distribution of information about plant suitability is of major importance.

Introduction

A key issue in the ecological success of leaf-cutting ants is their ability to exploit a broad variety of plant species. This polyphagism emerges through the intricate interplay between the ants and their symbiotic fungus. The ants are able to overcome mechanical plant defenses and their symbiotic fungus can cope with chemical defenses. Contrary to many other social insects, the harvested material brought back to the nest cannot be immediately utilized as food. Once the substrate reaches the nest, a complex process of preparation and incorporation into the fungus is necessary (Wilson 1971). First, the leaves are licked meticulously, then cut into pieces measuring 1-2 mm^2 and macerated until they are reduced to a pulp and finally incorporated into the fungus (Stahel, 1943; Quinlan & Cherrett, 1977; Herz et al. 2008). After this preparation, leaf fragments are inoculated with fungal cultivar (Mangone & Currie, 2007; Herz et al. 2008) and further tending of the fungus (Bass & Cherrett, 1994, 1996) yields specialized hyphal structures called gongylidia that are harvested as food (Weber 1972). Processing of leaf material inside the nest is an important component of fungus growing by attine ants. It occurs throughout most of the phylogenetic range and is present regardless of the substrate utilized (Mangone & Currie, 2007).

The specific tasks necessary during this process are carried out according to the body size of the workers (Weber, 1966; Wilson 1980). Wilson (1980) made a distinction between fixed tasks, which are attended by relatively limited age-size ensembles of workers, and flexible tasks, which are attended by ensembles that vary in size (and perhaps age) according to the objects treated during the performance of the task. The cutting of leaf fragments is an example for a flexible task were the physical characteristics, like different degrees of hardness and moisture contents, influence the size of workers performing the task (Camargo et al. 2003).

Three primary functions of this elaborate procedure processing the leaf material have been discussed so far. First, it provides the fungus with suitable physical conditions of the plant material increasing the ability of the fungus to break it down (Mueller et al. 1998). Second, the leaf processing plays an important part in nest hygiene as microbes are removed and the substrate is inoculated with mutualistic actinomycetous bacteria (Quinlan & Cherrett 1977; Mangone & Currie, 2007).

The third function of leaf processing, which has been rather neglected by research so far, serves the ants' need to evaluate the harvested substrates suitability and gain information about its effect on the fungus. Studies have shown that acceptance or rejection of a substrate by foragers in the field is influenced through a feedback of their symbiotic fungus (Ridley et al. 1996; North et al. 1999). This delayed rejection is species specific and occurs very fast, within 24 hours (Herz et al. 2008; Saverschek et al. 2010). Inside the nest, workers can gain information about the suitability of the harvested substrate from two sources: directly from the fungus and indirectly through other workers.

It has been postulated that a semiochemical from the fungus might be detected by the gardeners and passed on to the foragers ultimately changing their foraging behavior (North et al. 1999). This goes along with the material being processed and the fungus being cultured in an assembly-line fashion, with the succession of tasks being performed by ever smaller workers (Wilson 1980) and the fact that foragers change their foraging behavior through the influence of *experienced* gardeners alone (chapter 2). Then again, fungal changes inducing delayed rejection are likely to occur on a very small, localized scale in the range of square millimeters (Herz H, personal communication), even distribution of substrate throughout the nest takes 24-48 hours (Forti & Silveira Neto 1989; Pretto & Forti 2000; Moreira et al. 2003) and changes in the fungus are only detectable for 2-3 days (Herz et al. 2008). The retrievable information in the fungus is therefore patchy, temporally and spatially, and when it comes to efficiency of information flow inside the nest, a scenario where foragers involved in the leaf processing inside the nest can also detect and evaluate changes in the fungus themselves seems advantageous to the suggested channeling of information through the gardeners exclusively.

This study is the first to describe the behavior of gardeners and foragers towards processed substrate inside the fungus chambers against the background of the phenomenon of delayed rejection. I address the questions if the handling of substrate by workers is dependent on its suitability for the fungus and if there are differences between gardeners and foragers in their behavior towards the substrate. As I could already show that foragers can learn about suitability through gardeners (chapter 2), a special focus lay on ant-ant contacts. Specifically, I looked at the occurrence and duration of antennal contacts as means of information transfer. Ants learn about plant suitability through different sources depending on the amount of time that has passed since the first intake of the substrate in question (chapter 2). As time plays an important role, an additional experimental series was conducted in which the exposure to the unsuitable substrate lay in the past. Gardeners that had gained experience with the unsuitability of a substrate seven days previously were combined with *naïve* foragers and substrate that was now suitable. *Experienced* gardeners' behavior towards the substrate and other workers as well as the influence of their presence on the behavior of *naïve* foragers was observed to see if gardeners' experience had an influence.

Even though the existence of feedback from the fungus has been proven and a lot is known about the general time frame of rejection, little is known about the mechanisms underlying the observed patterns. This study tries to unravel some of the individuals' actions inside the nest that lead to the colony pattern observed on the outside.

Method

To better understand which underlying mechanisms allow foragers to learn about substrate suitability inside the nest, experiments with microcolonies were conducted to observe individual behavior directly within the fungus chamber. I could already show that substrate needs to be present in the fungus chamber to permit information transfer between the workers. Therefore the focus of observation lay on ant-plant contacts on the one hand as well as on ant-ant contacts on the other. Gardeners and foragers were marked individually and three different experimental series were conducted.

Microcolonies

Experiments were conducted with microcolonies obtained from 3 large lab colonies of *Acromyrmex ambiguus*. The colonies were collected in Uruguay in 2002 and maintained in the laboratory at 25°C under a LD cycle of 12:12 h. The set-up was comparable to that of the subcolonies (chapter 2), but on a smaller scale. Three Petri dishes (Ø 9 cm) connected by PVC-tubes served as an artificial nest. The center dish was filled more than half with fungus garden (fungus and gardening workers) and brood was added as a foraging stimulus for the microcolony. The dish serving as fungus chamber for the microcolony was then sealed with Parafilm© and connected to a refuse and a feeding dish. Approximately 100 foragers collected from the foraging trail were added to the refuse dish. The microcolonies were prepared at least 2 days previously to the start of the experiment to ensure well established fungus gardens and active foraging behavior. The microcolonies received fresh blackberry leaves (*Rubus fructicosus*) as well as water and honey water every day. The microcolonies did not receive any leaves the day before and the day of the experiment to keep the fungus garden free of any recent intake.

Experimental set-up

The established microcolony was placed under a binocular microscope connected to a camera (Figure 3.1). The camera was hooked up to a VCR and a monitor. Cold light was used to light up the 2 cm^2 observation area in the fungus chamber.

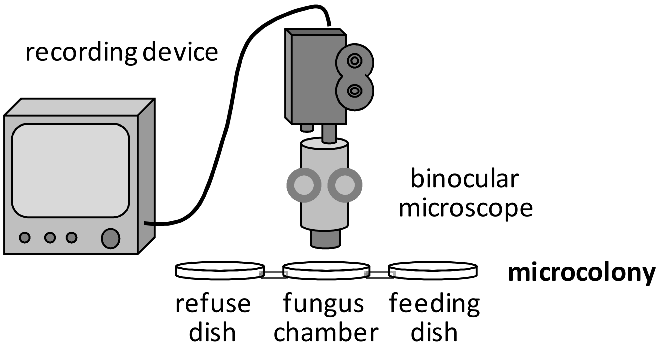

Figure 3.1: Microcolony in the experimental set-up. 2 cm^2-area of the fungus with freshly applied leaf patches was observed and the behavior of individually marked workers recorded.

Experimental procedure

Preparation of leaf material: The plant material added to the fungus chamber was processed to leaf pulp to make sure it would stay in the observed area and not taken to other areas of the fungus during the processing. Depending on the experimental series, a privet leaf was infiltrated with either water or a CHX-solution (procedure see chapter 1). The leaf was then cut into very small pieces (~1 mm^2), put into an Eppendorf© and stored in the freezer for 30 min.

Gardeners and foragers were distinguished by two criteria: body mass and location. Small workers collected directly from the surface of the fungus inside the fungus chamber were defined as gardeners whereas foragers were medium-sized and collected from the feeding dish. To make sure that visual judgment was accurate enough to obtain two distinct size groups a random sample of 79 gardeners and 99 foragers was weighed. There was a significant difference between the two groups of workers with gardeners weighing 2.6 mg ± 0.6 and foragers weighing 5.1 mg ± 1.1 ($p < 0.001$, $t = 18.29$, df = 176; unpaired t-test with independent variances).

Before the start of the observation, 60 foragers and 30 gardeners were ***individually marked*** with a 3x5 color code. Three dots of paint (Edding© 750) were carefully applied on head, thorax and abdomen with a bent paper clip. Marked workers were left in a separate box for several minutes to dry. No deleterious effect of the paint could be observed.

Preparation of microcolonies: All three Petri dishes were carefully opened and all foragers and as many of the gardeners as possible without harming the fungus were taken out. The leaf material was taken from the freezer, crushed and leaf pulp was applied with the tip of a needle to the surface of the fungus. Seven little patches were placed in an area of 2 cm^2 (observation area). 60 individually marked foragers and 30 individually marked gardeners were placed in the refuse dish and the whole microcolony was closed and sealed again. It was then placed under the binocular microscope and recording began 10 min later. As preliminary experiments had shown that the overall activity was considerably higher in the first than the third hour of observation, only the first hour of recording was analyzed.

Three different experimental series were conducted (Table 3.1). In the *control* series, *naïve* gardeners and foragers were added to a fungus with leaf patches consisting of suitable privet pulp. In the *treatment* series, *naïve* workers (gardeners and foragers) were added to a fungus with privet pulp that contained cycloheximide and was therefore unsuitable for the fungus.

Table 3.1: Overview of the three experimental series conducted. Behavior of marked individuals was observed in three microcolonies per series and data was pooled.

experimental series	**gardeners**	**foragers**	**leaf patches (pulp)**	**n (microcolonies)**
control	*naïve*	*naïve*	suitable	3
treatment	*naïve*	*naïve*	unsuitable	3
experience	*experienced*	*naïve*	suitable	3

In the *experience* series, microcolonies received 10 leaf disks of privet infiltrated with CHX (see chapter 1) seven days previously to the start of the experiment. On the day of the experiment, *naïve* foragers were added to the fungus containing *experienced* gardeners and freshly applied leaf patches of untreated, suitable privet pulp. The recorded videos were analyzed focusing on two areas of interest:

Ant-plant contacts: two distinct behaviors could be identified. Ants approached the leaf patch, antennated it very briefly and moved on quickly, often not even really stopping. These contacts lasted only 1 second or less and are referred to as antennating the leaf patches. The second type of ant-plant contact, from now on referred to as handling the leaf patch, consisted of workers approaching the leaf patch, antennating, licking and sometimes biting it. The ant-plant contacts were recorded on all 7 leaf patches and pooled.

Ant-ant contacts: ants frequently antennated others, sometimes head-to-head, sometimes one individual drumming with its antennae on any body part of another individual. If the contact was clearly initiated by one of the partners, the initiating ant was recorded. Ant-ant contacts were recorded in the whole observation area (2 cm^2), irrespective of leaf patches.

Data analysis

Three microcolonies per experimental series were tested. Data was pooled for each series. The occurrence, frequency and duration of all behaviors were recorded. The sum of all gardeners (foragers) showing one of the observed behaviors was calculated as percentage of all marked gardeners (foragers) added to the microcolonies and is referred to as active gardeners (foragers). This activity level was used to standardize the number of workers showing recorded behavior across experimental series. Differences between experimental series or between gardeners and foragers within series were tested with chi^2-tests and Mann-Whitney-U-tests.

Results

The influence of substrates' suitability on the behavior of gardeners and foragers was investigated comparing *control* and *treatment* series.

Gardeners

First, the general activity of gardeners in both experimental series was compared. Activity level was the same in both series with 41.1 % of all gardeners active in the *control* series and 40.0 % active in the *treatment* series (p = 0.88, chi^2 = 0.02).

Ant-plant contacts: A high percentage of active gardeners, over 80 %, contacted the leaf patches in both control and treatment (Figure 3.2; p = 0.80, chi^2 = 0.06).

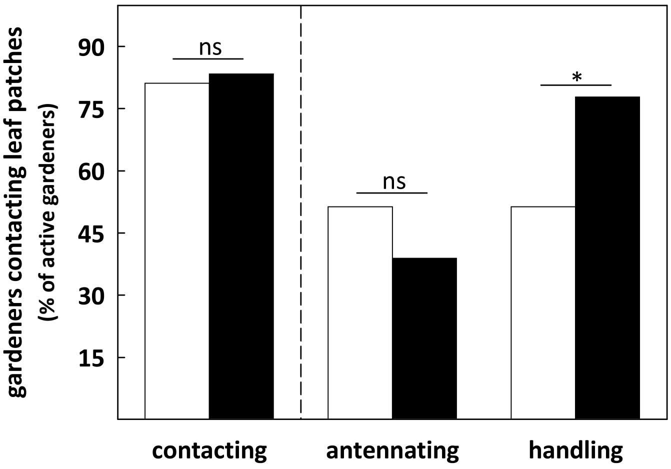

Figure 3.2: Percentage of active gardeners contacting leaf patches in *control* (☐) and *treatment* (■). Contacting = antennating and/or handling leaf patches; antennating = briefly antennating leaf patches; handling = antennating, licking and sometimes biting leaf patches. n (*con*/*treat*) = 37/36. Significance of chi^2-tests is given as ns = non significant and *p < 0.05.

There was no significant difference in the percentage of active gardeners briefly antennating the leaf patches between the two experimental series (p = 0.28, chi^2 = 1.14). However, the percentage of active gardeners handling the leaf patches was significantly higher in the *treatment* series (77.8 %) compared to the *control* series (51.4 %; p = 0.02, chi^2 = 5.56). In the *control* series, gardeners handled the leaf patches around 5 seconds (± 3), whereas in the *treatment* series, gardeners handled the leaf patches on average three times as long (17 ± 29 s). Due to a high variance of durations in the *treatment* series and the non-normal distribution of the data however, there was no significant difference in handling times between *control* and *treatment* (p = 0.96, U = 263.00, Z = -0.05).

To have a closer look at the occurrence of different handling times within each experimental series, the distribution was plotted (Figure 3.3). There was a significant difference between *control* and *treatment* in the percentage of gardeners handling leaf patches longer than 11 seconds (p= 0.02, chi^2 = 5.58).

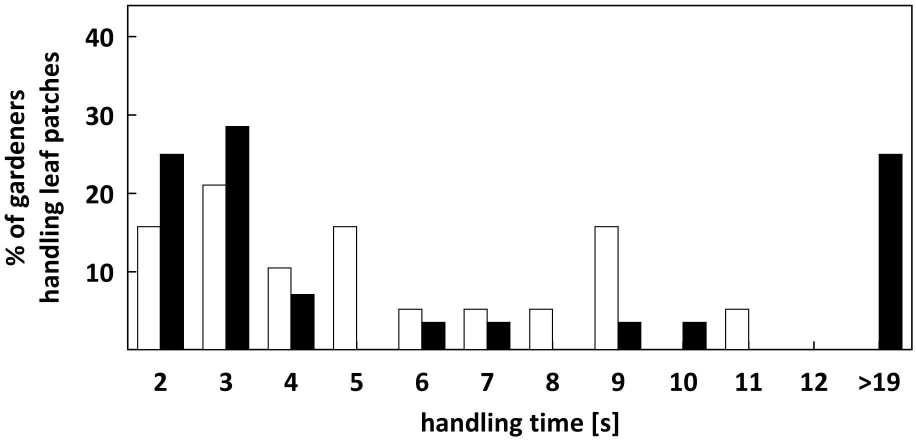

Figure 3.3: Distribution of gardeners' average handling time [s] of leaf patches in *control* (☐) and *treatment* (■). n (*con/treat*) = 19/28.

Handling time of leaf patches in the *control* series ranged from 2 to 11 seconds at the most. In the *treatment* series on the other hand, 25 % of all gardeners had an average handling time of more than 19 seconds. The longest average duration of a gardener handling unsuitable leaf patches was 106 seconds.

Ant-ant contacts: Significantly more gardeners were engaged in ant-ant contacts when the substrate was unsuitable than when the substrate was suitable (Figure 3.4).

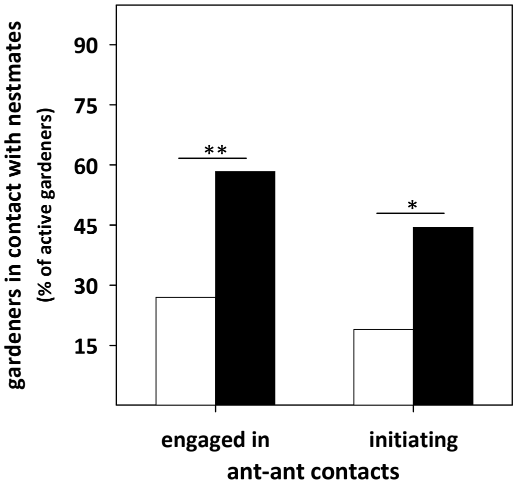

Figure 3.4: Percentage of active gardeners engaged in ant-ant contacts and initiating ant-ant contacts in *control* (☐) and *treatment* (■). n (*con*/*treat*) = 37/36. Significance of chi^2-tests is given as *p < 0.05 and **p < 0.01.

Percentage of gardeners engaged in contacts was twice as high when leaf patches were unsuitable (58.3 %) than when they were suitable (27.0 %; $p < 0.01$, $chi^2 = 7.32$). The data was then further sorted according to who initiated the contact, regardless of the receiving ant being a gardener or forager. Again, the percentage of gardeners was significantly higher in the *treatment* series than in the *control* with 44.4 % of gardeners initiating contacts in the *treatment* series, almost twice as many as in the *control* series (18.9 %; $p = 0.02$, $chi^2 = 5.51$).

Looking at the number of times individual gardeners initiated contacts with other workers there was a significant difference between the two series (Figure 3.5; *top*).

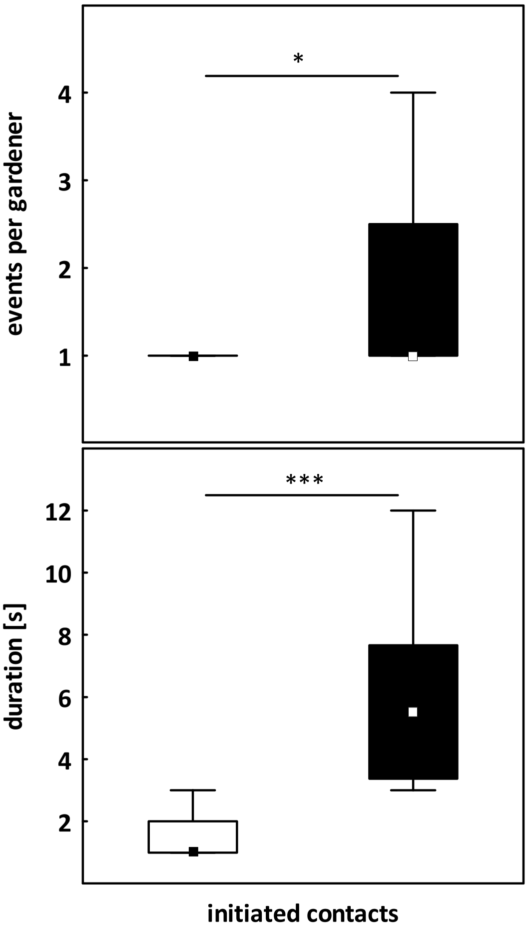

Figure 3.5: Number of initiated contacts per gardener (*top*) and average duration [s] of initiated contacts (*bottom*) in *control* (☐) and *treatment* (■). n (*con*/*treat*): = 7/16. Data is shown as median, quartile range and range. Significance of MWU-tests is given as *p < 0.05 and ***p < 0.001.

In the *treatment* series gardeners initiated 1-3 contacts on average, whereas gardeners in the *control* series initiated only one contact per individual ($p < 0.05$, $U = 31.5$, $Z = -1.60$). The duration of contacts was significantly different in the two experimental series (Figure 3.5; *bottom*). Contacts initiated by gardeners lasted 1-2 seconds in the *control* series, significantly shorter than in the *treatment* series, where gardener-ant contacts lasted between 3 to 7 seconds ($p < 0.001$, $U = 1.5$, $Z = -3.61$).

Foragers

Whereas the general activity of gardeners was similar in both experimental series, the percentage of active foragers significantly differed between series ($p < 0.001$; $chi^2 = 53.94$). In the *control* series, more than twice as many foragers were active (62.2 %) than in the *treatment* series (23.9 %).

Ant-plant contacts: The percentage of active foragers contacting the leaf patches lay between 75 - 85 % and was independent of plant suitability (Figure 3.6; $p = 0.25$, $chi^2 = 1.35$). There was also no significant difference between the percentages of active foragers antennating the leaf patches in both experimental series (~ 60 %; $p = 0.94$, $chi^2 = 0.00$). The percentage of foragers handling the leaf patches in *control* and *treatment* was also not significantly different, with 38.4 % in the *control* series and slightly more48.8 % in the *treatment* series ($p = 0.24$, $chi^2 = 1.40$).

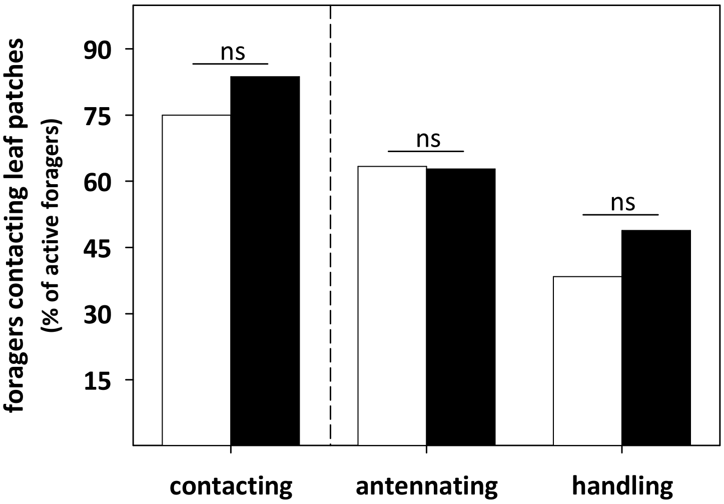

Figure 3.6: Percentage of active foragers contacting leaf patches in *control* (☐) and *treatment* (■). Contacting = antennating and/or handling leaf patches; antennating = briefly antennating leaf patches; handling = antennating, licking and sometimes biting leaf patches. n (*con/treat*) = 112/43. Significance of chi^2-tests is given as ns = non significant and *p < 0.05.

Looking at the handling time, foragers handled the leaf patches around 4 (± 2) seconds in the *control* series. In the *treatment* series, foragers handled the leaf patches on average 30 (± 71) seconds. Due to a high variance of durations in the *treatment* series and the non-normal distribution of the data, there was no significant difference in handling times between *control* and *treatment* though ($p = 0.21$, $U = 364.00$, $Z = -1.24$).

To have a more detailed look at the occurrence of different handling times within each experimental series, the distribution was plotted (Figure 3.7).

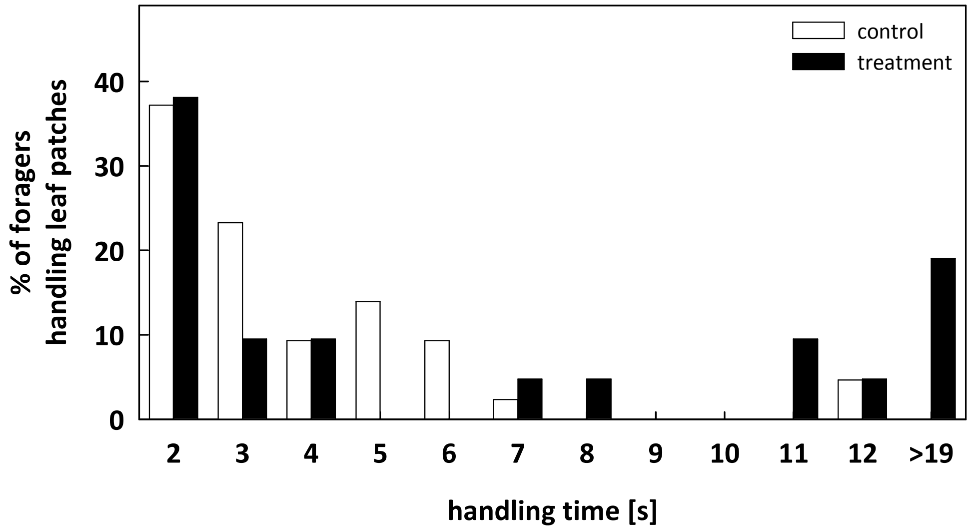

Figure 3.7: Distribution of foragers' average handling time [s] of leaf patches in both series. n (*control* ☐ / *treatment* ■) = 43/21.

In the *control* series, no forager handled leaf patches longer than 12 seconds on average, whereas in the *treatment* series, 19.0 % of all foragers handled the leaf patches longer than 19 seconds on average ($p < 0.01$, $chi^2 = 8.74$).

Ant-ant contacts: In the *treatment* series 62.8 % of active foragers engaged in ant-ant contacts, whereas significantly less foragers, only 33.9 %, engaged in contacts in the *control* series (Figure 3.8; $p \leq 0.001$, $chi^2 = 10.63$).

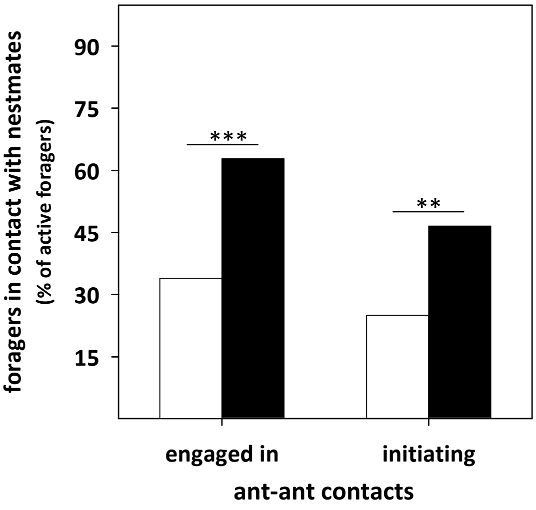

Figure 3.8: Percentage of active foragers engaged in ant-ant contacts or initiating ant-ant contacts in *control* (☐) and *treatment* (■). n (*con*/ *treat*) = 112/43. Significance of chi^2-tests is given as $^{**}p < 0.01$ and $^{***}p \leq 0.001$.

Looking only at the foragers that initiated contacts, irrespective of the ant they contacted, forager or gardener, the percentage was significantly higher in the *treatment* series than in the *control* ($p < 0.01$, $chi^2 = 6.73$). Almost twice as many foragers (46.5 %) initiated contacts in the presence of unsuitable leaf patches compared to 25 % in the *control* series with suitable leaf patches applied on the fungus.

Foragers initiated contacts 1-2 times per individual irrespective of the suitability of the substrate present (Figure 3.9; *top*).

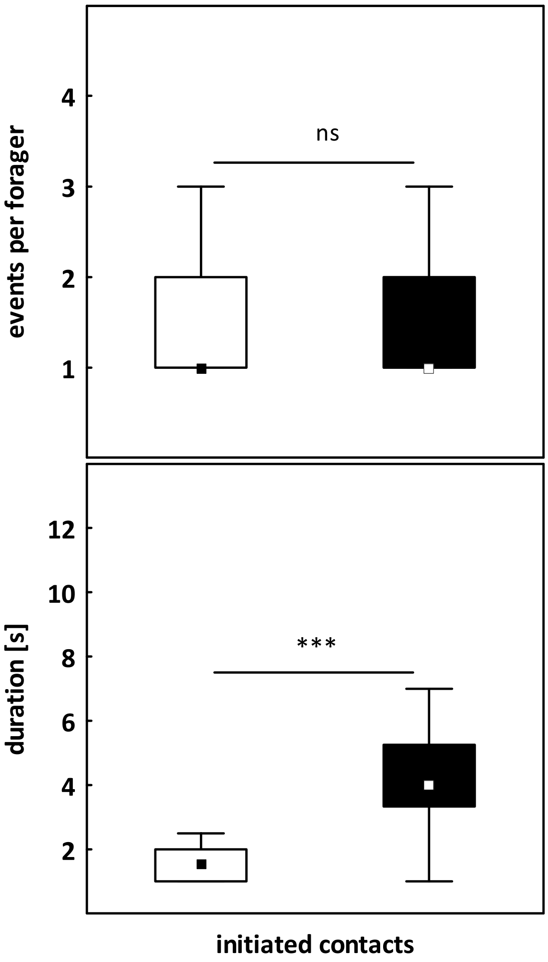

Figure 3.9: Number of initiated contacts per forager (*top*) and average duration [s] of initiated contacts (*bottom*) in *control* (☐) and *treatment* (■). n (*con/treat*) = 28/20; Data is shown as median, quartile range and range. Significance of MWU-tests is given as ns = non significant and ***p < 0.001.

There was no significant difference between the two experimental series ($p = 0.91$, $U = 274.00$, $Z = 0.12$). The duration of contacts was significantly higher in the *treatment* than the *control* series (Figure 3.9; *bottom*). In the presence of unsuitable substrate, contacts initiated by foragers lasted 4-6 seconds whereas in the presence of suitable substrate, the contacts lasted 1-2 seconds ($p < 0.001$, $U = 36.00$, $Z = -5.09$).

Gardeners and foragers: Comparison within each experimental series

In the *control* series, a significantly higher percentage of foragers (62.2 %) were active compared to the percentage of active gardeners (41.1 %). Besides the difference in general activity ($p = 0.001$; $chi^2 = 10.81$), gardeners and foragers showed no difference in occurrence, frequency or duration of observed behaviors.

In the *treatment* series, general activity of the two worker groups was exactly the opposite from the *control* series with a significantly smaller percentage of foragers (23.9 %) being active in comparison to active gardeners (40.0 %; $p < 0.01$, $chi^2 = 7.52$). When the plant was unsuitable for the fungus, ant-plant contacts also differed significantly between gardeners and foragers. Percentage of gardeners antennating leaf patches (38.9 %) was significantly lower ($p < 0.05$, $chi^2 = 4.48$) in comparison to foragers (62.8 %), whereas the percentage of gardeners handling the leaf patches (77.8 %) was significantly higher than the percentage of foragers (48.8 %) handling leaf patches ($p < 0.01$, $chi^2 = 6.97$). There was no difference in the frequency or duration of the ant-plant contacts. There was also no difference in the ant-ant contacts between gardeners and foragers.

Experience series

The transfer experiments (chapter 2) showed that the presence of *experienced* gardeners in the nest alone is enough to lower the acceptance of a substrate by foragers in the field, even if they encounter suitable leaf disks in the fungus garden. In the *experience* series, the focus lay on the information transfer between gardeners and foragers of different experiential background. *Naïve* foragers and suitable substrate were added to a fungus garden containing *experienced* gardeners that had encountered unsuitable substrate 7 days previously. The gardeners in this experimental series were exposed to the same factors as the ones in the *control* series – suitable substrate and *naïve* foragers only that they had previous 'negative' experience with the now suitable substrate. The foragers were exposed to two differing information sources about the suitability of the substrate: leaf patches with suitable substrate on the one hand and gardeners with 'negative' experience regarding that substrate on the other.

Results from the *experience* series were compared with the *control* series, to see if gardeners' experience with the unsuitability of a substrate in the past, seven days previously has an impact on the behavior of gardeners and foragers towards the now suitable substrate and if it influences ant-ant contacting behavior (Table 3.2).

Table 3.2: Overview of general activity and behaviors recorded in the *control* and *experience* series. To test for statistical significance MWU-tests were used; chi^2-test was used for percentages.

	gardeners			**foragers**		
	control	***experience***		***control***	***experience***	
no. of active ants (% of marked ants)	37 (41.1)	32 (35.6)	ns	**112 (62.2)**	**34 (18.9)**	**p < 0.001**
ant-plant contacts						
antennating leaf patches [%]	**51.4**	**78.1**	**p < 0.05**	63.4	67.6	ns
handling of leaf patches [%]	51.4	46.9	ns	38.4	38.2	ns
events per ant median (Q_1/Q_3)	2.0 (1-2)	2.0 (1-4)	ns	1.0 (1-2)	2.0 (1-3)	ns
duration [s] median (Q_1/Q_3)	4.5 (2.7-7.5)	4.0 (2-4.5)	ns	3.0 (2-5)	2.8 (2-4.3)	ns
>19 sec [%]	0.0	13.3	ns	0.0	0.0	ns
ant-ant contacts						
initiating contacts [%]	18.9	18.8	ns	25.0	20.6	ns
events per ant median (Q_1/Q_3)	1.0 (1-1)	1.0 (1-1)	ns	1.0 (1-2)	2.0 (1-3)	ns
duration [s] median (Q_1/Q_3)	**1.0 (1-2)**	**3.0 (2-5)**	**p < 0.05**	**1.5 (1-2)**	**2.5 (2-5)**	**p < 0.01**

There was no significant difference in the general activity of gardeners in the *control* (41.1 %) compared to the *experience* series (35.6 %; p = 0.44, chi^2 = 0.59). Looking at the foragers, who themselves were *naïve* towards the substrate, but in the company of *experienced* gardeners, differences in activity in comparison with the *control* series could be found. The general activity of foragers was significantly lower in the *experience* series than in the *control* series (p < 0.001, chi^2 = 70.10).

Ant-plant contacts: Significantly more gardeners (78.1 %) briefly antennated the untreated leaf patches in the *experienced* series than in the *control* series (51.4 %; p = 0.02, chi^2 = 5.32). The previous negative experience with the substrate had no effect on the occurrence, frequency or duration of the handling time of the leaf patches. There were no differences in the foragers' behavior towards the leaf patches. Occurrence, frequency and duration of ant-plant contacts were comparable to the *control* series.

Ant-ant contacts: Looking at the contacts initiated by gardeners in the *experience* series, there was no difference in the percentage of gardeners initiating contacts, nor in the number of times individuals initiated contacts, but a significant difference in the duration (Figure 3.10).

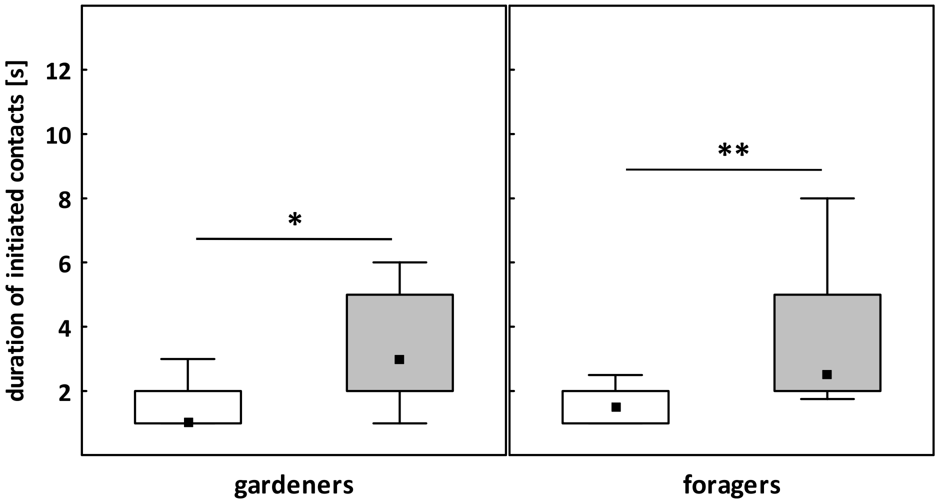

Figure 3.10: Average duration of initiated contacts [s] in the two experimental series, *control* (☐) and *experience* (■). (forager (*con*/*exp*): n = 28/7; gardener (*con*/*exp*): n = 7/6). Data is shown as median, quartile range and range. Significance of MWU-tests is given as *p < 0.05 and **p < 0.01.

Contacts initiated by gardeners that had previous ‘negative’ experience with the suitable substrate lasted 2-5 seconds, significantly longer than contacts initiated by gardeners without previous experience in the *control* series with an average of 1-2 seconds.

Interestingly, the duration of contacts initiated by foragers was also significantly longer in the *experience* series (2-5 s) than in the *control* series (1-2 s) and just as long as the contacts initiated by the gardeners, even though they had no negative previous experience concerning the substrate. There was no difference between contacts initiated by gardeners or foragers in the *experience* series.

Discussion

Information about the suitability of harvested substrate is a key element in the efficiency of the foraging process of social insects. In leaf-cutting ants, the situation is especially complex as the substrate they harvest serves to culture their symbiotic fungus. After it reaches the nest, the substrate first needs to be processed and incorporated into the fungus before ants can evaluate the substrates’ suitability from its effects on the fungus. Substrates are distributed evenly throughout the fungus chambers and incorporated into the fungus on a very small scale. Leaf-cutting ants are very sensitive to changes occurring in their fungus and therefore able to assess the substrates’ effect on the fungus locally (*Acromyrmex lundi,* Herz et al. 2008).

Influence of the substrates’ suitability for the fungus on the behavior of gardeners and foragers inside the fungus chamber

Gardeners show significant changes in their ***behavior towards the processed leaf patches*** dependent on the suitability of said substrate. Even though the general activity stays the same (around 40 %), the percentage of gardeners handling the leaf patches, licking and sometimes biting it, is higher if the substrate is unsuitable. 25 % of all gardeners handling the unsuitable substrate proceed longer than 19 seconds if it is unsuitable, whereas suitable leaf patches are never handled longer than 11 seconds on average. The percentage of active gardeners briefly antennating the leaf patches is independent of the suitability of the substrate.

Foragers are significantly less active in the presence of unsuitable substrate, but there is no difference in the percentage of active foragers’ antennating or handling the leaf patches in control or treatment. Like the gardeners, a significant percentage of foragers handling the leaf patches (19 %) perform the task longer than 19 seconds if the substrate is unsuitable though. Suitable leaf patches are never handled longer than 12 seconds on average.

Leaf-cutting ants are able to differentiate between plants through their specific odor alone (chapter 1) and the percentage of active ants antennating the leaf patches is the same in both experimental series. This leads to the conclusion that a brief antennation of the plant pulp is enough to identify the substrate and might be sufficient to establish if a freshly processed substrate is suitable for the fungus or not. As the unsuitable substrate was treated with a fungicide undetectable by the ants (Ridley et al. 1996), ants must be able to detect effects on the fungus itself. If the leaf patch is evaluated as unsuitable, the percentage of active gardeners handling the leaf patches is higher and a significant percentage of both gardeners and foragers handle the leaf patches longer. This is in accordance with findings in workers of *Acromyrmex octospinosus* who are known to lick leaf fragments sprayed with an arrestive solution much longer than untreated leaf disks during the first steps of the preparation process of the plant material in the nest (Quinlan & Cherrett 1977).

The suitability of the substrate for the fungus not only influences the behavior of ants towards the leaf patches, but also has significant effects on ***ant-ant contacts*** in close vicinity of the processed substrate. If the substrate is unsuitable, a higher percentage of gardeners as well as foragers initiate contacts. Additionally, the number of times an individual gardener initiates a contact with another ant is also higher, when the substrate is unsuitable. Considering that choice of substrate and its suitability for their symbiotic fungus is of critical importance for the colony, it is important to distribute information about the unsuitability of an already harvested and processed substrate. Workers can gain or spread information through interactions with other workers (Pratt 2005; Greene & Gordon 2007; O'Donnell & Bulova 2007) and antennal communication as a mean of transferring communication has been shown in several ant species before (Lenoir 1982; Jaffe & Villegas 1985). The increasing rates of ant-ant contacts initiated by gardeners as well as foragers could therefore be a sign of increasing distribution of information about the unsuitability of the substrate. In order to encode this information in antennal communication, the substrate in question needs to be present during the contact. I could already show that transfer of information between workers about the unsuitability of substrates is only possible in the presence of identifiable substrate inside the fungus chambers (chapter 2). As observed antennal contacts were not accompanied by trophallaxis, workers might associate the information received through antennation with the odor of the substrate present. All ant-ant contacts were recorded within the 2 cm^2 observation area of the fungus, the only area in the fungus chamber on which the processed substrate was present. Foragers of *Acromyrmex ambiguus* are able to distinguish between plant odors from a distance of at least 2 cm during foraging (chapter 1). As seven little leaf patches were applied within this small area, it can safely be assumed that workers could detect and identify the plant odor during ant-ant contacts.

Not only did the overall occurrence of ant-ant contacts increase, but also the duration of contacts was significantly longer if the substrate was unsuitable. This is a strong indication that the duration of ant-ant contacts encodes information about the suitability of the processed substrate present in the fungus. The duration of antennal contacts as a possible code for information about the profitability of a source has already been described during trophallaxis in honeybees were longer contacts were observed when there was a high solution transfer-rate (Goyret & Farina 2003).

Differences in the observed behavior of gardeners and foragers

Experiments with *Atta sexdens* have indicated that contact between smaller workers may be sufficient to pass information concerning the suitability of fungal substrate to foragers (North et al. 1999). In fact, in *Acromyrmex ambiguus*, information about substrate suitability can not only be retrieved from the fungus itself, but also conveyed to *naïve* workers by *experienced* ones inside the nest. *Naïve* foragers are able to learn about the unsuitability of a substrate through *experienced* gardeners even in the absence of the harmful effects of said substrate on the fungus (chapter 2). As the substrate is processed and the fungus cultured in an assembly-line fashion, with the succession of tasks being performed by ever smaller workers (Wilson 1980), it has been postulated that the information about substrate suitability might also flow in an assembly-line fashion with the smaller workers (gardeners) being more sensitive towards changes in the fungus. As gardeners could not yet be experimentally excluded from the fungus without causing considerable harm to it, it is still unknown if they are a necessary link between fungus and foragers.

The extent of foragers’ tasks inside the fungus chambers are not fully understood yet. They are part of the first steps of preparing and processing the substrate for its incorporation into the fungus (Wilson 1980), but it is still unclear how much they are part of the incorporation and pruning of the leaf material later on in the process. In the control series, when the processed substrate present in the fungus is suitable, there is no difference in the behavior of gardeners and foragers towards the substrate. At least throughout the time period of our observation, when the substrate is already processed, but not yet incorporated into the fungus, gardeners and foragers perform the same set of tasks inside the fungus chamber. In the treatment series though, were the substrate is unsuitable, there are significant differences in the percentage of active ants antennating or handling the leaf patches. A significantly higher percentage of active foragers only check the leaf patches briefly through antennation, whereas a significantly higher percentage of gardeners handle the leaf patches. Even though a lower percentage of active foragers compared to gardeners handle the leaf patches in the treatment series, a significant percentage of those foragers handling the unsuitable leaf patches still shows extended handling times in comparison to the handling of suitable leaf patches. Therefore it is highly likely that foragers are able to gain the same information as gardeners from the fungus.

In large societies, like leaf-cutting ant colonies, it is advantageous for the distribution of information throughout the colony for foragers to have two opportunities to gain information about substrate suitability inside the nest: a direct collection of information through evaluation of the effects the substrate has on the fungus and indirectly via other already informed workers.

The general activity of gardeners and foragers is significantly different in both treatments. Whereas gardeners have an activity level of about 40 % in both experimental series, the percentage of active foragers is significantly higher in the control series (62 %) and significantly lower in the treatment series (24 %). The lower activity of foragers if the substrate is unsuitable leads to the conclusion that they might only assist in incorporating the substrate into the fungus if it is suitable. If it is unsuitable gardeners primarily handle the leaf patches performing the tasks of cleaning the fungus or pruning it to increase productivity.

Influence of experience

The presence of *experienced* gardeners influences *naïve* foragers' plant preferences even in the absence of effects of the plant species in question on the fungus (chapter 2). The *experience series* was conducted to find behavioral patterns that might shed some light on the way the information about the unsuitability of a plant is transferred from *experienced* gardeners to *naïve* foragers in the presence of suitable samples of said plant.

Ant-plant contacts are influenced by previous experience of gardeners. The percentage of active gardeners briefly antennating the leaf patches was significantly higher in the experience series than the control series. Foragers in the field are able to recognize substrate through odor alone and associate previous negative experience with it (chapter 1) so it seems likely that gardeners are also able to do the same inside the nest. Gardeners recognize the odor of the substrate and therefore the probability of them approaching the patches increases. Interestingly though, the percentage of gardeners handling the leaf patches does not increase neither does the handling time. This strengthens the conclusion that they recognize the substrate, remember its previous unsuitability and therefore are more likely to approach and antennate the leaf patches briefly. When they notice the suitability of the substrate, handling becomes shorter and less likely, comparable to the control series. Forager-plant contacts are not influenced by the presence of *experienced* gardeners neither in occurrence nor duration. As the substrate itself is suitable, this might indicate that foragers are able to evaluate substrate suitability and its effect on the fungus themselves.

Interestingly, the duration of ***ant-ant contacts*** initiated by gardeners or foragers is also influenced by the negative experience of gardeners. *Experienced* gardeners initiate significantly longer contacts in the *experimental series* than in the *control series* even though the substrate now present in the fungus is suitable. *Experienced* gardeners seem to rely on their assessment of the plants identity through brief antennation, associate that with their experience with the plant and therefore initiate long contacts comparable to the situation with unsuitable substrate present.

This quick assessment of the substrates identity and suitability is prone to leading to an overreaction, an unnecessary rejection of a now suitable substrate, but this response also prevents the further intake of substrate previously encountered as unsuitable as fast as possible. If *experienced* gardeners ‘overreacted’, foragers of such subcolonies reject the substrate on their next foraging cycle. If some now suitable substrate reaches the fungus garden, acceptance increases again 48 hours later (personal observation). Surprisingly, *naïve* foragers also initiate these long contacts with other workers when the substrate is suitable if they are in the company of *experienced* gardeners. So the presence of gardeners with a negative experience regarding the substrate in question is enough to stimulate foragers to also initiate significantly longer contacts with other workers.

The general activity of gardeners is similar in both experimental series (35-40 %), whereas the general activity of foragers is significantly lower in the presence of *experienced* gardeners in comparison to the control series. This further strengthens the assumption that the behavior of the *experienced* gardeners present leads the foragers to believe that the substrate is unsuitable.

It seems like both ways of information flow from the fungus to the foragers directly and indirectly from the fungus via the gardeners to the foragers operate simultaneously. Based on the rather similar behavior of gardeners and foragers towards the leaf patches in both experimental series and the significant differences in forager-plant contacts between suitable and unsuitable leaf patches it seems that foragers are able to directly evaluate the effects of the substrate on the fungus. Taking the results of the *experience series* as well as the results from the transfer experiments (chapter 2) into account, it is evident that *experienced* gardeners have significant influence on the behavior of foragers inside and outside the nest. The significantly lower general activity of foragers in the treatment as well as the experience series might indicate that the ant-ant contacts initiated by gardeners might dampen the foragers’ readiness to perform tasks in the fungus chamber.

CHAPTER 4

MODULATION OF INDIVIDUAL PLANT PREFERENCES THROUGH SOCIAL INFORMATION

Abstract

Leaf-cutting ant colonies harvest a broad variety of substrates for their symbiotic fungus. As several plant species are harvested simultaneously on different trails, foragers differ not only in their innate tendencies, but also in their individual experiences with certain substrates which leads to different plant preferences of individuals within the colony. Foraging together on long, highly populated trails creates an environment in which social information about plant suitability is present. This study shows the influence of social information on substrate selection of leaf-cutting ant colonies. Comparing plant preferences of individuals foraging alone or in a group, there is no difference if foragers choose between two plant species that are both familiar and suitable. If one of the two offered suitable plant species is unfamiliar though, individuals foraging on their own significantly preferred the familiar plant species, whereas workers foraging in a group accepted both plant species equally well. As foragers are influenced in their substrate choice through their nestmates, social information increases the acceptance of new substrates. Choosing between two familiar plant species, one known as unsuitable, one as suitable, significantly more foragers still accept the unsuitable substrate when foraging on their own compared to foraging in a group. It is known that foragers learn about the unsuitability of a substrate through their symbiotic fungus within 24 hours. My results lead to the conclusion that foragers also gain information about the unsuitability of a substrate indirectly through nestmates on the trail. Foraging in a group and the presence of social information is therefore a decisive factor in the substrate choice of individuals leading to a consentaneous and distinct colony response when encountering unfamiliar or unsuitable substrate.

Introduction

Unraveling the influencing factors on the decision-making process of individuals is important in order to understand animal behavior. Foraging is a major component of animal behavior and animals being able to adjust their foraging decisions to new or changing environments increase their fitness (Pyke 1984).

In social insects, the simple chain of cause and effect of solitary animals' foraging decisions is not viable. Decisions made by individuals have effects not only on the individual level, but on the colony level as well. Foraging decisions of individuals are primarily based on innate tendencies that even vary within the same colony (*Acromyrmex octospinosus*, Therrien 1988) which might lead to different plant preferences among individuals based on differences in perception and evaluation of plant characteristics. Several food sources are depleted simultaneously in different locations and colonies consist of tens of thousands of workers and several fungus chambers (Weber 1972) leading to different experiential backgrounds of foragers within a colony (*Atta colombica*, Saverschek et al. 2010). As leaf-cutting ants forage together, variation in plant preferences among workers creates an environment in which social information might be another factor influencing the behavior of individuals.

Danchin et al. (2004) categorize available information into (1) personal information and (2) social information. Personal information is acquired through interactions of individuals with their physical environment. Social information is present through the presence of other individuals, their behavior and impact on the environment or each other. Social information can be based on signals, defined as traits specifically shaped by natural selection to convey information (also see Seeley 1989) or cues that arise incidentally by individuals engaged in the performance of their task.

In leaf-cutting ants, the social environment of an individual consists not only of nestmates, but also of the symbiotic fungus that plays an important role in the regulation of foraging. Ants receive feedback about the suitability of the harvested substrate from their symbiotic fungus and adjust their plant preferences accordingly (Ridley et al. 1996, North et al. 1999). Whether the information about plant suitability is accessible through a cue that ants perceive in the fungus or if the fungus emits a signal to actively inform the foragers about the suitability of the harvested substrate, still remains to be investigated. But information about plant suitability is not only available directly from the fungus. Foragers can also learn inside the fungus chambers through the presence of ants that already had experience with the substrate in question (chapter 2) and recorded contact rates among ants imply that experienced ants might actively influence naïve workers (chapter 3).

Probably the most impressive example for social learning is the honey bee dance. Foragers perform a succession of stereotypical motions inside the hive signaling nestmates the information about the location of food sources (von Frisch 1967). Contrary to honeybees, leaf-cutting ants gain experience about host plants moving together on densely populated trails where individuals are influences by social signals like pheromones and acquire information through presence of nestmates (Jaffe & Howse 1979). Besides information about the location of food sources, foragers' plant preferences are also influenced on the way to the food source. In the context of recruiting, *Acromyrmex lundi* workers are conditioned to the odor of the food fragment carried by the scout worker and used the learned cue to decide which material should be collected at the food source (Roces 1990) and recruited workers of *Atta colombica* clearly preferred resources encountered on trails during their outward journey (Howard et al. 1996). The question arises if individuals foraging in a group make different, maybe more accurate foraging decisions due to social information available on the way to the food source.

Indications for interactions between foragers on the trail influencing individual foraging decisions have already been found in field studies with *Atta colombica* (Saverschek et al. 2010). Acceptance of a formerly unsuitable plant species did not occur linearly as one might expect assuming a gradual replacement of old experienced workers through new naïve workers in the foraging column, but on the contrary, resumption of acceptance occurred from one acceptance test to the next. Such a rather sudden change in acceptance might be an indication of workers influencing each other during the collective harvesting activity.

In this study, foragers' preferences were compared between foraging individually or in a group in order to investigate possible changes in individual foraging decisions. Does social information change plant preference? Is there a difference in plant preference between the sum of individual choices and group choice? In other words, does the presence of nestmates, inadvertently through cues or directly through signals, influence foragers on the trail resulting in an emergent foraging pattern of the colony that is more than the sum of its parts? As suitability and familiarity of plant species as well as differences among the experiential background of foragers are suspected to play a role, three experimental series were conducted. Foragers were familiar with the tested plant species in the first two experimental series. First, two suitable plant species were presented simultaneously (*suitable* series) and in the *unsuitable* series foragers had to choose between two plant species one suitable and one unsuitable for their symbiotic fungus. In the third experimental series (*unfamiliar* series), two suitable plant species were offered, one familiar and the other one unfamiliar to foragers in order to investigate if individual differences in innate tendencies of foragers to choose a certain substrate would cause differences in foraging decisions on the group level.

Methods

To see if there is a difference between foraging alone or in a group in the decision-making process of the individual, pairings of two plant species were offered to individuals and groups of foragers.

Subcolonies

Experiments were conducted with subcolonies obtained from 5 large lab colonies of *Acromyrmex ambiguus*. The colonies were collected in Uruguay in 2002 and maintained in the laboratory at 25°C and under a LD cycle of 12:12 h. Artificial nests consisted of three transparent Plexiglas®-boxes connected by short PVC-tubes (10 cm, Ø 3 cm). The center box (19 x 8.5 x 8.5 cm) served as the fungus chamber and the two other boxes (19 x 19 x 8.5 cm) as feeding-box and refuse-box respectively. The bottom of the fungus-box was covered with moistened expanded clay pebbles to keep the humidity high and prevent desiccation of the fungus. The lids of the refuse- and feeding-box had three holes each (Ø 3 cm), covered with a fine metallic mesh allowing air circulation to create a different, dryer microclimate from the one inside the fungus-box, an *inside* and *outside* for the subcolony.

About 1000 ml of fungus garden (fungus and gardening workers) were taken from the mother colony and placed in the artificial nest together with approximately 1000 foragers. Subcolonies were established at least 4 days prior to the start of the experiment to ensure well established fungus gardens and active foraging behavior. Subcolonies received fresh rose leaves (*Rosa canina*) and water every day and honey water every other day.

Experimental set-ups

The feeding-box of the subcolony was disconnected and replaced with a PVC-tube (20 cm, Ø 1.2 cm) leading directly from the fungus chamber to the entry platform (Figure 4.1).

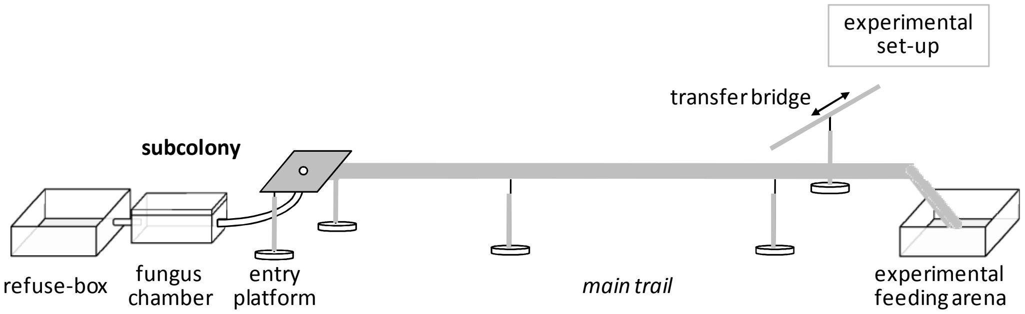

Figure 4.1: General set-up. A wooden bridge connects the entry platform with the experimental feeding arena 1.5 m away. The transfer bridge to single out individual foragers to the experimental set-up was placed 1 m away from the entry platform and 50 cm away from the experimental feeding arena.

From this platform, a 5 cm wide and 1.5 m long wooden bridge (*main trail*) lead to another box (19 x 19 x 8.5 cm) used as experimental feeding arena. A movable side element, the *transfer* *bridge*, (10 x 0.5 cm) could be connected to the *main trail* halfway between the standard feeding arena and the experimental feeding arena. The transfer bridge led to the experimental set-up.

The ***individual set-up*** consisted of a rectangular platform (2 x 4 cm) with a toothpick (3 cm) attached in the center of the long side (Figure 4.2, *left*). One leaf disk of each tested plant species was placed on the platform equidistantly from the entering point. The ***group set-up*** consisted of a square platform (10 x 10 cm) on which 20 leaf disks (10 of each plant species) were distributed randomly (Figure 4.2, *right*). Foragers entered the platform via the transfer bridge.

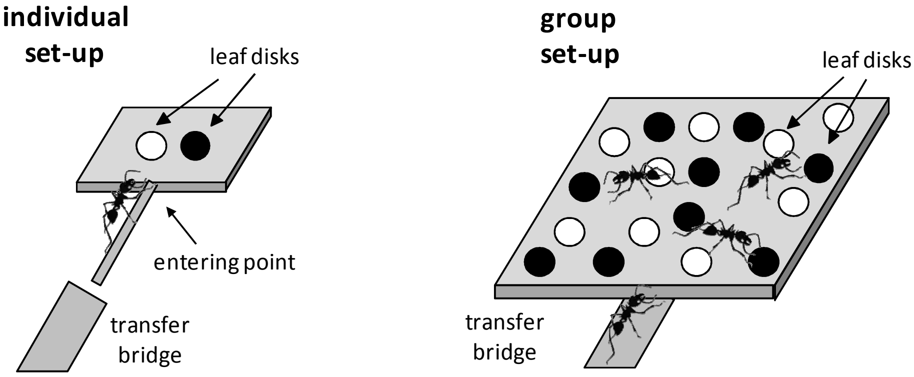

Figure 4.2: Experimental set-ups to test foragers individually and as a group. Individual set-up (*left*): individual foragers could access a platform (2 x 4 cm) with the two tested plant species offered as leaf disks equidistantly from the entering point of the platform. Group set-up (*right*): from the main trail across the transfer bridge, foragers could freely access the platform where 10 leaf disks of each tested plant species were offered simultaneously.

Experimental procedure

Three experimental series were conducted, each with both set-ups (individual and group). The reference plant in each series was blackberry, a familiar and suitable plant species that had been the main food source of the colonies for several weeks before the experiments (Table 4.1).

Table 4.1: Overview of all experimental series and tested plant pairs. The three experimental series were conducted with both set-ups (individual and group).

experimental series				**tested plant pairs**
suitable	suitable familiar	vs.	suitable familiar	privet vs. blackberry
unsuitable	unsuitable familiar	vs.	suitable familiar	privet vs. blackberry
unfamiliar	suitable unfamiliar	vs.	suitable familiar	plum vs. blackberry

In the *suitable* series, foragers chose between two familiar, suitable plant species (privet and blackberry). Privet had also been fed to the subcolonies regularly several weeks before the experiments. The *unsuitable* series was conducted after the treatment of the subcolonies with fungicidal privet, so the acceptance of privet as a now unsuitable, familiar plant simultaneously offered with blackberry, familiar and suitable, was tested. In the *unfamiliar* series, the acceptance of a suitable, but unfamiliar plant species which had not been fed to the subcolonies for at least six months (plum) was tested in a preference test with the familiar and suitable blackberry.

To guarantee well established foraging, subcolonies foraged along the *main trail* for at least 2 days previous to the experiment. They only received fresh rose leaves in the experimental feeding arena before and during experiments. On the day of the experiment, the colony was given time (between 20-30 min) to establish the foraging process along the main foraging trail, until a constant number of laden foragers returned from the feeding arena. During this time period, the transfer bridge was placed on the *main trail* to ensure pheromone markings on it. Pheromone markings increased the percentage of ants leaving the *main trail* and walking along the transfer bridge.

In the ***individual experiment***, the transfer bridge was used to single out foragers from the main trail. Once a forager had entered the bridge, it was carefully moved towards the experimental set-up leaving a gap of several centimeters towards the main trail so no other foragers could follow. The forager then entered the platform via the toothpick encountering a leaf disk of each of the two tested plant species on it. The foragers' decision was recorded when it left the platform with one of the leaf disks. In between foragers, the platform and its leaf disks were replaced to avoid any influence through potential pheromone traces. Position of the two plant species was alternated to account for possible side preferences of the foragers. If an individual did not make a decision within 2 minutes, the test was discontinued and the next forager was tested. In the ***group experiment***, leaf disks of both plant species were offered *ad libitum* i.e. picked up leaf disks were immediately replaced with the same plant species. Leaf disks (Ø 6 mm) were always punched out freshly before each experiment. Once the platform was connected with the *main trail* via the transfer bridge foragers could access the platform freely and a preference test was conducted for 10 minutes. The first 40 ants to return from the platform during this time frame carrying a leaf disk were recorded. After the *suitable* series (two familiar, suitable plant species), before conduction of the *unsuitable* series, the subcolonies received fungicidal leaf disks of privet to create a negative experience with this plant species.

Treatment of plant material: An aqueous solution of cycloheximide (CHX; Sigma-Aldrich®, Deisenhofen, Germany) was used. This fungicide is undetectable by the ants but has been proven to be a potent fungicide to the attine fungus in the laboratory (Ridley et al. 1996, North et al. 1999, Herz et al. 2008). In order to maintain leaf specific properties such as odor, surface characteristics and toughness, the leaf internal airspace was infiltrated with an aqueous solution (0.03 %, w/w) of cycloheximide (see Herz et al. 2008). Each subcolony received 110 leaf disks of the treated plant species. The experimental series 3 was then conducted 24 hours later.

Data analysis

Data of 40 successfully tested individuals, i.e. foragers that made a decision within 2 min in the experimental set-up, was collected per subcolony with 8 subcolonies in the individual experiments. One group test per subcolony was performed with 8 subcolonies in the group experiments. To comply with requirements of normality all values were arcsine transformed prior to statistical analyses. Significance of difference in acceptance from random distribution was tested with t-test for single mean. Significant differences in acceptance between individual and group experiments were tested with non-paired t-tests.

Results

Two suitable, familiar plant species, privet and blackberry, were offered in the *suitable* series (Figure 4.3). Individuals chose both plant species equally, regardless if they foraged alone (p = 0.15, t = -1.62, df = 7) or in a group (p = 0.95, t = -0.06, df = 7).

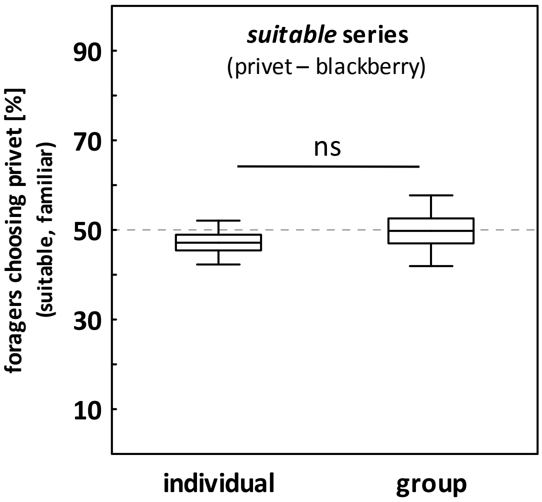

Figure 4.3: Comparison of preferences between foragers choosing individually or in a group between two suitable plant species: familiar (privet) versus familiar (blackberry). Data are mean ± SE, and n = 8 subcolonies for each group (with N = 40 individuals tested per subcolony). Significance of paired t-tests of acceptance within each group before and after the treatment of the subcolonies is given as ns.

There was no difference in the exhibited plant preferences due to the presence or absence of nestmates throughout foraging (p = 0.44, t = 0.80, df = 14). Before conduction of the *unsuitable* series, subcolonies received treated privet leaf disks to create a negative experience with this plant species. 24 hours after the treatment, foragers' plant preferences were tested with leaf disks of privet and blackberry in both set-ups (Figure 4.4).

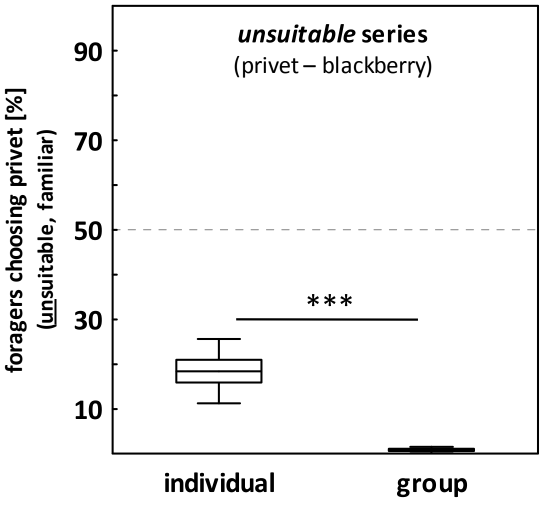

Figure 4.4: Comparison of preferences between foragers choosing individually or in a group between two familiar plant species: unsuitable (privet) versus suitable (blackberry). Data are mean ± SE, and n = 8 subcolonies for each group (with N = 40 individuals tested per subcolony). Significance of paired t-tests of acceptance within each group before and after the treatment of the subcolonies is given as ***P < 0.001.

Acceptance of privet, now known as unsuitable to the treated subcolonies, was extremely low in individual as well as group tests. Foragers tested individually showed a very low acceptance of privet (p < 0.001, t = -10.16, df = 7). Foragers choosing between both plant species in the presence of other nestmates also rejected privet almost completely (p < 0.001, t = -37.43, df = 7). Even though foragers strongly rejected privet in both experiments, foragers deciding in the absence of their nestmates still accepted privet significantly more often than foragers choosing in a group (p < 001, t = 9.16, df = 14).

In the *unfamiliar* series, foragers were confronted with two suitable plant species, one unfamiliar (plum) and one familiar (blackberry; Figure 4.5).

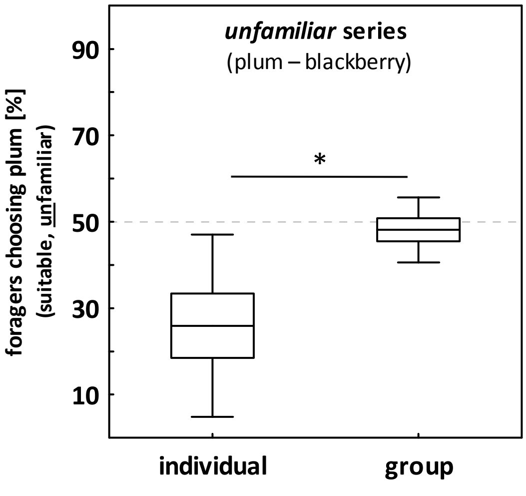

Figure 4.5: Comparison of preferences between foragers choosing in a group or individually between two suitable plant species: unfamiliar (plum) versus familiar (blackberry). Data are mean ± SE, and n = 8 subcolonies for each group (with N = 40 individuals tested per subcolony). Significance of non-paired t-tests of acceptance within each group before and after the treatment of the subcolonies is given as *P < 0.05.

Individual foragers clearly preferred the familiar plant species over the unfamiliar, with only 20-30 % of tested individuals deciding on plum (p = 0.02, t = 3.19, df = 7). Ants foraging in a group on the other hand showed no significant preference for either of them (Figure 4.5; p = 0.50, t = 0.71, df = 7).Comparing the preferences between foragers choosing plant species in a group to foragers deciding by themselves showed a significant difference (p = 0.01, t = 2.84, df = 14; Welch's t-test for uneven variances).

Discussion

In a diverse and seasonal environment, polyphagus herbivores have to cope with changing suitability and availability of host plants throughout their lifetime. It is therefore of utmost importance to them to constantly monitor and evaluate potential host plants. Leaf-cutting ants evaluate substrates based on innate tendencies and physical and chemical plant characteristics (Stradling 1978, Cherrett & Seaforth 1970, Hubbell et al. 1983). Their preferences are also influenced by the feedback of their symbiotic fungus (Ridley et al. 1996; North et al. 1999; Herz et al. 2008; Saverschek et al. 2010). Foragers learn about substrate suitability and adjust their host plant preferences accordingly.

In social insects, there are two types of acquiring information: personal information, that is collected through interactions with the environment and social information gained through observing the behavior of others towards the environment (Danchin et al. 2004). Several studies have already highlighted some of the personal information leaf-cutting ants use to assess the suitability of a potential host plant, but the question if individuals’ plant preferences might also be influenced through social information, i.e. the presence of nestmates interacting with their environment is only partly answered. This study specifically targeted the differences between the presence and absence of potential social information during substrate selection at the foraging site under three different conditions.

When ***both offered plant species are familiar and suitable*** to the subcolony, preferences are the same regardless if foragers decide alone or in a group context. Foragers accepted blackberry and privet equally well and showed no significant preference for either of the two plant species. In the individual set-up, foragers chose between plant species assessing physical and chemical properties of the offered leaf disks and evaluating them based on their innate tendencies and personal experience. It is known that individual foragers of the same colony can exhibit differing plant preferences probably due to different innate tendencies or different experiential background (*Acromyrmex octospinosus*, Therrien 1988). As all foragers had previous positive experience with both substrates, group foraging provided no further information about plant suitability for the individual.

If ***one of the two suitable plant species is unfamiliar***, the situation is quite different. In that case, foragers' preferences significantly differ between foraging alone compared to foraging in a group. Ants foraging individually significantly preferred blackberry, the familiar substrate. Only 25 % chose the unfamiliar plant species plum. This might either be due to the fact that blackberry was assessed as the more suitable plant of the two or the fact that foragers already had previous experience with the substrate. Experiments with *Atta colombica* have shown that scout ants cut pieces and initiated recruitment significantly earlier when encountering familiar resources than when encountering new resources (Howard et al. 1996). This might be due to the complex decision-making system in leaf-cutting ants where the second level of quality control occurs through the symbiotic fungus inside the nest. Reluctance towards new food sources might therefore be beneficial as unfamiliar plant species assessed as suitable by foragers might still prove to be unsuitable for the fungus. Colonies of *Atta colombica* recruiting to a newly discovered potential host plant show moderate foraging activity on the day of the discovery and increase foraging efforts on the second day if the substrate has proven to be suitable for the fungus (Saverschek 2004).

When ants were foraging in a group, blackberry and plum were accepted equally. Innate tendencies as a reason for the observed preference for blackberry by foragers in the individual set-up can therefore be excluded. 50 % of foragers chose the unfamiliar plant species, twice as many as in the individual set-up. This discrepancy in preferences can be explained through the influence of social information on the decision-making process of individuals. Foragers seem to be only reluctant towards the unfamiliar plant species plum when foraging alone. Foraging in a group exposes foragers to the plant preferences of their nestmates and foragers deciding on the new substrate might influence others either through active encouragement or, more likely, inadvertently through their presence on the trail carrying the new substrate. These results are in accordance with experiments conducted on field colonies of *Atta colombica*, where foragers clearly preferred resources encountered on trails during their outward journey (Howard et al. 1996). In the context of recruiting, foragers of the grass-cutting ant *Acromyrmex lundi* tend to choose substrates carried by a scout they have encountered on the way to the food source (Roces 1990, 1994). Foragers' acceptance of an unfamiliar substrate when it seems to be suitable based on personal information is further increased by social information obtained through the presence of other foragers carrying the substrate. Social influence accelerates the acceptance of new substrates as foragers are conditioned to plant species carried by nestmates encountered on their way to the food source (Howard et al. 1996). Foragers are influenced by the behavior of other nestmates and the collective pattern emerging is therefore shaped by the additional information available through foraging in a group. A distinction can be made between acquisition of information via cues or signals (Seeley 1989). Whereas signals have the primary function of communicating information, cues occur incidentally. Whether information about the suitability of the unfamiliar plant is communicated actively during foraging or if foragers are inadvertently influenced through their nestmates behavior remains elusive.

When presented with ***two familiar substrates, one suitable and one unsuitable***, foragers also show significantly different preferences dependent on if they forage individually or in a group. 24 hours after the treatment of the subcolony with fungicidal privet leaf disks, 20 % of ants foraging individually still preferred privet over blackberry indicating a lack of information about the plant's unsuitability. The unsuitability of a substrate is mediated through the symbiotic fungus and foragers are able to change their preferences within 24 hours (Herz et al. 2008; Saverschek et al. 2010). Field studies have shown a time delay in the distribution of information about the unsuitability of a harvested substrate through experienced workers throughout the colony leading initially to different levels of acceptance on different trails (*Atta cephalotes*, Ridley et al. 1996; *Atta colombica*, Saverschek et al. 2010). Even though subcolonies in this study consisted only of one fungus chamber and approximately 1000 foragers, not every individual seemed to be informed yet about the plant's unsuitability for the symbiotic fungus.

Foraging in a group, the acceptance of privet was significantly lower with only 0-2 % of foragers still accepting the unsuitable plant species. This leads to the conclusion that foragers might also gain information about the plants' unsuitability throughout the foraging process. In the previous situation described, a cue i.e. presence of nestmates on the trail carrying the unfamiliar substrate could be already be enough to increase its acceptance by foragers encountering it on the way to the food source. It seems unlikely that this could also work the other way round with a lack of foragers carrying the unsuitable substrate influencing the decision of foragers lacking experience with it. Information about the unsuitability of the substrate is more possibly spread actively throughout the group, either on the trail or directly during plant selection at the food source. Active signaling is always associated with costs e.g. trade-off between information transfer and size of carried leaf disk during initial recruitment in leaf-cutting ants (*Acromyrmex lundi*, Roces & Núñez 1993). It is conceivable nonetheless, as the rapid identification and rejection of unsuitable substrate is of utmost importance to the colony to keep negative effects on their symbiotic fungus minimal.

This study shows that individual plant preferences are modulated through information perceived in a social context. Group foraging facilitates social learning leading to a consensus on the suitability of a substrate among foragers.

CHAPTER 5

INFLUENCE OF EXPERIENCED FORAGERS DURING FORAGING ON PLANT PREFERENCES OF NAÏVE NESTMATES

Abstract

Foragers harvest plants not for themselves, but for their symbiotic fungus which in turn influences their preferences through feed-back after the incorporation of the harvested material. As foraging trails of mature colonies are up to 100 m long and foragers therefore spend a considerable amount of time away from the nest it seems conceivable that information about the suitability of a substrate might also be communicated directly on the trail between workers of different experiential background. This study shows that several sources of information lead to a decrease in acceptance of formerly unsuitable substrate by naïve foragers within one foraging day. If effects of the substrate on the fungus are still detectable, naive foragers change their acceptance within four hours irrespective of the presence or absence of experienced foragers on the trail. In the absence of a cue in the fungus, the presence of experienced foragers has a significant influence on the point in time when a change in acceptance by naïve foragers occurs. In absence of experienced foragers, with information only being available indirectly through experienced gardeners inside the nest, naïve foragers change their acceptance within six hours. If experienced foragers as well as experienced gardeners were present, naïve foragers also decreased their acceptance within four hours already even though there were no effects of the substrate on the fungus detectable directly anymore. This shows that naïve foragers decreased their acceptance of a formerly unsuitable substrate within the same time period irrespective of the source of information, whether directly through detecting detrimental effects on the fungus or indirectly through the presence of experienced foragers and gardeners. The average percentage of experienced foragers on the trail without a load correlated with the level of rejection of the formerly unsuitable substrate by naïve foragers after eight hours of foraging whereas no correlation with the percentage of laden experienced foragers could be found. Unladen experienced foragers might actively contact laden naïve workers transmitting information about the unsuitability of the load they carry. Even though in the laboratory, the effect of experienced foragers on the trail on the acceptance of substrate by naïve foragers is superimposed by the feedback from the symbiotic fungus it has a significant effect in the absence of the fungal cue. Out in the field, the influence of experienced foragers on naive foragers might be of importance even if the cue in the fungus is still present as foraging trails are considerably longer than in the laboratory and naïve foragers return to the nest less frequently.

Introduction

Foraging decisions of leaf-cutting ants are a multi-layered process, as evaluation of host plants by individuals is not only based on the foragers' innate tendencies, but also influenced by their symbiotic fungus, previous experience and the behavior of their nestmates.

Leaf-cutting ants are a popular model of central-place foraging (Wilson 1980; Rudolph & Loudon 1986; Roces 1990; Kacelnik 1993; Burd 2000) with the nest serving as center of information. Foragers return to the nest with their load and harvested leaf fragments are then processed to serve as substrate for the cultivation of their mutualistic fungus (Mueller et al. 1998). Previous studies have shown that colonies of leaf-cutting ants are able to adjust their foraging preferences within 24 hours, if substrate proves to be unsuitable for their fungal symbiont (Ridley et al. 1996; North et al. 1999; Herz et al. 2008; Saverschek et al. 2010). Several days after the incorporation of unsuitable substrate into the fungus, when a cue is no longer present, foragers are still able to learn about the unsuitability of the substrate inside the nest through the presence of experienced nestmates (chapter 2). This suggests that experienced foragers might also be able to inform nestmates about a substrate's unsuitability away from the nest.

Leaf-cutting ants forage along an extensive trail system consisting of permanent, well-established trunk trails branching out to currently harvested host plants (Weber 1972). Conditioning of workers on the trail has already been shown in the context of recruiting. Foragers of *Acromyrmex lundi* are influenced in their substrate choice through odor of the load carried by a scout they encounter on the way to the newly discovered food source (Roces 1990, 1994). In *Atta cephalotes,* one contact with a laden ant on the way to the new food source can be enough to stimulate an unladen ant to look for a food item and increases its probability to carry it to the nest (Farji-Brener et al. 2010). Howard et al. (1996) postulated conditioning of foragers of *Atta colombica* to the plant species making up the majority of loads encountered along the way to established food sources. In the above studies, foragers were positively influenced in their decisions through encounters with laden nestmates returning to the nest on the trail.

It is still unknown if information about unsuitability of plants for the fungus which are not detectable by ants directly might also be distributed through social interactions on the trail, but several factors make it seem likely. Even though the effects of unsuitable substrate on the fungus seem to be detectable within a few hours after the incorporation, information about a substrates' unsuitability is not evenly distributed throughout large colonies within 24 hours (Ridley et al. 1996; Saverschek et al. 2010). Complete rejection of the substrate can be observed on the trail where the substrate was harvested within 24 hours, and adjacent trails show complete rejection 2-3 days after incorporation of the substrate. This suggests that the experiential background of foragers regarding the unsuitable substrate differs for 2-3 days. Workers of *A. colombica* exhibit low trail fidelity, with approximately 15-45 % of media workers changing trails within 24 hours (Porter & Bowers 1982; Wagner 2004). This leads to a mixture of foragers with different experiential background on the trails, workers that are either naïve or experienced regarding a certain substrate.

As foragers spend a considerable amount of time outside the nest on trails throughout their daily foraging bouts it seems conceivable that information about plant suitability might be communicated not only inside the nest, but also outside on the trails throughout the harvesting process. It seems highly adaptive that information about plants that damage the symbiotic fungus spreads quickly throughout the colony.

This study tries to elucidate the questions if preferences of *naïve* foragers, without previous experience of the unsuitable substrate, are influenced through the presence or absence of *experienced* foragers on the trail during foraging. As foragers frequently return to the nest between foraging trips, the situation inside the fungus chambers also needed to be put into consideration. Influence of experienced foragers was therefore tested under two different conditions, either with or without the cue from the fungus still present inside the nest.

Methods

To see if foragers can learn about the unsuitability of a substrate already throughout one foraging day, *naïve* foragers were given the opportunity to continuously forage for eight hours under four different conditions. *Naïve* foragers were transferred either alone or with a majority of *experienced* foragers to fungus gardens one day or seven days after the incorporation of unsuitable substrate (privet) i.e. with or without the cue about the unsuitability of privet present in the fungus garden. To look at the dynamic of acceptance, preference tests were conducted regularly throughout the eight hours of a daily foraging cycle.

Subcolonies

Experiments were conducted with subcolonies obtained from 5 large lab colonies of *Acromyrmex ambiguus*. The colonies were collected in Uruguay in 2002 and maintained in the laboratory at 25°C and under a LD cycle of 12:12 h. Artificial nests consisted of three transparent Plexiglas®-

boxes connected by short PVC-tubes (10 cm, Ø 3 cm). The center box (19 x 8.5 x 8.5 cm) served as the fungus chamber and the two other boxes (19 x 19 x 8.5 cm) as feeding-box and refuse-box respectively. The bottom of the fungus-box was covered with moistened expanded clay pebbles to keep the humidity high and prevent desiccation of the fungus. The lids of the refuse- and feeding-box had three holes each (Ø 3 cm), covered with a fine metallic mesh allowing air circulation to create a different, dryer microclimate from the one inside the fungus-box, an *inside* and *outside* for the subcolony.

About 1000 ml of fungus garden (fungus and gardening workers) were taken from the mother colony and placed in the artificial nest together with approximately 1000 foragers. Subcolonies were established at least 4 days prior to the start of the experiment to ensure well established fungus gardens and active foraging behavior. The subcolonies received fresh blackberry leaves (*Rubus fructicosus*) and water every day and honey water every other day.

Experimental set-up

The subcolony was connected to a wooden ramp that led out of the standard feeding box onto a 5 cm wide and 4 m long wooden bridge (*main trail*) leading to a wooden platform (10 x 10 cm) were food was provide and preference tests were conducted (see Figure 5.1). A camera was installed above the bridge halfway between the subcolony and the platform. Subcolonies foraged along the main trail for at least 2 days before the start of the experiment.

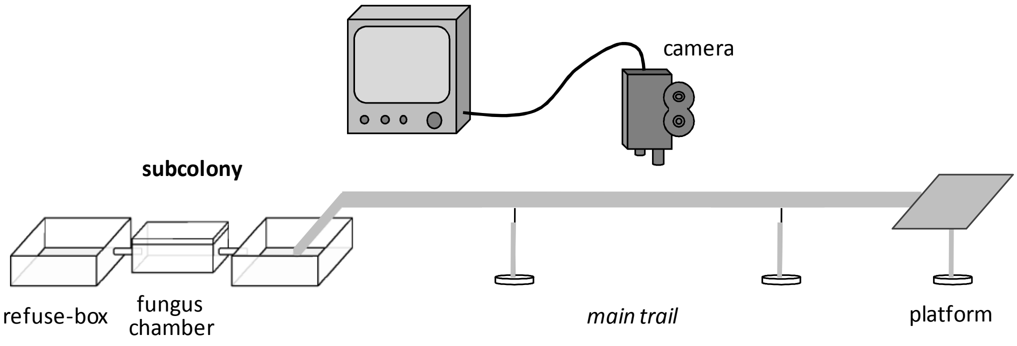

Figure 5.1: Experimental set-up. A ramp led foragers on a bridge which connected the subcolony to a platform 4 m away were substrate was presented. A camera was installed above the bridge halfway between the subcolony and the platform.

Experimental procedure

Standardized preference tests were used throughout the course of the experiment to measure the acceptance of privet by foragers. The procedure was always the same. The subcolony was given time (between 20-30 min) to establish the foraging process along the main foraging trail collecting blackberry leaves. The preference test was conducted as soon as a constant number of laden foragers returned from the platform the colony. Leaf disks (Ø 6 mm) of privet and blackberry (20 each) were then offered simultaneously instead of the blackberry leaves. Whenever a leaf disk was picked up and carried off the platform, it was immediately replaced by a leaf disk of the same type (*ad libitum*). Foragers leaving the platform with their load were recorded. The test was conducted for 25 min.

Treatment of subcolonies: to create different experiential backgrounds, subcolonies were exposed to one of two treatments. They either received 110 untreated, suitable leaf disks of privet (from now on: *naïve* subcolonies) or 110 leaf disks of treated, unsuitable privet (from now on: *experienced* subcolonies). The treated leaf disks were infiltrated with CHX (see methods, chapter 1) and offered to subcolonies together with untreated blackberry leaf disks (Figure 5.2, *before treatment*).

Transfers of foragers to a different fungus garden took place on day 1 or day 7 after treatment (Figure 5.2). Before transfer, a preference test (*before transfer*) was conducted to verify the status quo of the subcolonies after the treatment.

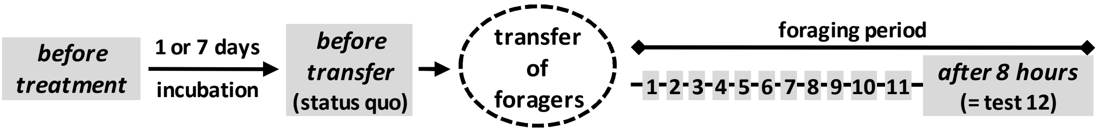

Figure 5.2: General diagram for all experimental series. Timeline of preference tests conducted in all four experimental series.

Studies with *Acromyrmex lundi* have shown that naive foragers were only able to retrieve the information about the unsuitability of a substrate if they were transferred within 2 days after the incorporation of the substrate (Herz et al. 2008). Day 1 and 7 after the treatment were chosen to have experienced fungus gardens with and without the effects of the unsuitable substrate on the fungus still detectable i.e. with and without cue. Then all foragers were collected from the subcolony, so that only the fungus with its gardening workers remained (*fungus garden*). In order to retrieve the foragers from the artificial nest, the fungus-box was separated from the refuse- and feeding-box and all workers outside the fungus-box were collected. Foragers tend to come out of the garden when the nest is disturbed. The lid of the fungus-box was also lifted and large individuals were carefully collected of the top of the fungus with featherweight forceps. The whole procedure did not exceed 45 min and the fungus-box was kept shut as much as possible to minimize desiccation of the fungus. Once all foragers had been collected from the fungus, the fungus-box containing the fungus and its gardeners was connected again with the empty feeding- and refuse-box.

700 foragers were then added to the refuse-box and the artificial nest was closed again. Foragers were added to the refuse box to ensure that any forager that appeared on the main trail had passed the fungus garden. The subcolony was given time (20 min) to reestablish the foraging process along the main trail. The 12 preference tests were conducted on the platform at the end of the main trail every 40 min over a period of 8 hours. In between preference tests, blackberry leaves were offered on the platform to provide an unlimited food source throughout the whole course of the experiment and dropped leaf disks of privet and blackberry in front of the nest entrance were collected to (1) keep foraging activity high and to (2) limit the amount of untreated privet entering the fungus garden.

One control and four experimental series were conducted (Table 5.1). To exclude the possibility of a decrease in the acceptance of privet throughout the eight hours of foraging due to factors not considered in the experimental design (Roces 1990; Howard et al. 1996; chapter 1 & 4) and to quantify the dynamics, a control series was conducted. It also served as a baseline for comparison.

Table 5.1: **Experimental series.** One control and four experimental series were conducted with *naïve* and *experienced* foragers transferred to subcolonies at two different times after fungus treatment.

experimental series	**time of transfer**	**fungus garden**		**foragers**	**n**
		fungus	**gardeners**		
control	day 1	-	naïve	only naïve	8
I	day 1	cue	experienced	naïve & experienced	6
II	day 1	cue	experienced	only naïve	7
III	day 7	no cue	experienced	naïve & experienced	6
IV	day 7	no cue	experienced	only naïve	5

It is known that *naïve* foragers without foraging experience can gain information about substrate suitability inside the nest within 24 hours (North et al. 1999; Herz et al. 2008; chapter 2). Here the question was addressed if *naïve* foragers learn about substrate suitability throughout the daily foraging bouts. The influence of presence and absence of *experienced* foragers on substrate preferences of *naïve* foragers was investigated under two different conditions. In series I and II, the cue about the unsuitability of the substrate was still present in the fungus whereas in series III and series IV, there was no cue in the fungus anymore.

Depending on the series, transferred foragers were only *naïve* (control, II & IV) or a combination of *naïve* and *experienced* (I & III). If *naïve* and *experienced* foragers were transferred together, the ratio was always 200 *naïve* foragers versus a majority of 500 *experienced* foragers.

If a mixture of *naïve* and *experienced* foragers was added to a subcolony, one group of ***foragers was marked*** with a dot of paint (Edding® 750) on the thorax. The paint was carefully applied with the tip of a bent paper clip. Marked workers were left in a separate box for several minutes to dry before they were put back in the colony. No deleterious effect of the paint could be observed.

Data analysis

Observer 2.0®, a DOS-based program for observational data collection (Noldus Information Technology, Wageningen, Netherlands) was used to record the observations from the preference tests.

The ***standardized acceptance of privet*** was calculated by dividing the number of privet leaf disks picked up and taken away through the number of all leaf disks that were picked up and taken throughout the preference test (25 min). Values for standardized acceptance therefore ranged from 0.0 to 1.0 with a value of 0.5 indicating equal acceptance of both offered plant species. Differences in acceptance of privet from *before transfer* to *after 8 hours* were compared in all experimental series. To comply with requirements of normality, all values were arcsine transformed prior to statistical analyses. Standardized acceptance of privet of *naïve* and *experienced* subcolonies were compared using unpaired t-tests. Differences in acceptance between *before transfer* and *after 8 hours* were tested with paired t-tests in for each experimental series.

Change in acceptance between *before transfer* and *after 8 hours* was calculated as follows and compared across experimental series. Acceptance of privet by *naïve* subcolonies minus the acceptance of privet by their paired *experienced* subcolonies *before transfer* was defined as 100 %. Acceptance of privet *after 8 hours* was calculated as percentage of these. A square root transformation was used prior to statistical analyses to comply with requirements of normality and differences in changes of acceptance between different experimental series were tested using unpaired t-tests.

Foraging activity throughout the experiment was measured using camera recordings. Traffic was counted for three minutes each 10, 15 and 20 min after the start of each preference test. Inbound and outbound ants were counted distinguishing between laden and unladen foragers and, depending on the experimental series, between *naïve* and *experienced* foragers. Mean number of foragers per minute was calculated for each preference test and each category.

Results

Before the start of the experimental series, the experiential background of all subcolonies in regards to the acceptance of privet was compared between the two different treatments. Subcolonies that had received untreated privet leaf disks (n = 32) showed a standardized acceptance of privet of 0.41 ± 0.12 (mean ± SE) and were therefore considered *naïve* regarding possible negative effects of privet on their fungus. Subcolonies that had received fungicidal leaf disks (n = 24) accepted privet significantly less (0.04 ± 0.06, mean ± SE) than *naïve* subcolonies in preference tests before the transfer and were therefore considered *experienced* ($p \leq 0.001$, t = 14.55, df = 54).

Acceptance of privet in the control series

To test if the acceptance of privet would be constant or if, how it would change over an eight hour foraging period, a control experiment was conducted in which *naïve* foragers were transferred to a *naïve* fungus garden (Figure 5.3). Acceptance of untreated privet offered with blackberry did not decrease over the course of the experiment (p = 0.17, t = -1.52, df = 7) but was slightly higher though not significantly than in the beginning of the experiment.

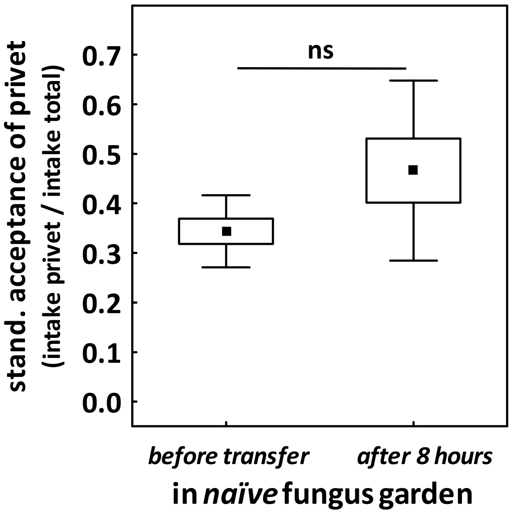

Figure 5.3: Acceptance of privet by *naïve* foragers transferred to a *naïve* fungus garden 1 day after treatment (control). 700 *naïve* foragers were transferred and acceptance of privet was compared between *before transfer* and *after 8 hrs* of foraging. Data are mean ± SE with n = 8. Significance of paired t-test comparing *naïve* foragers *before transfer* and *after 8 hours* is given as ns = non significant.

Influence of experienced foragers in the presence of a cue in the fungus

If there is a cue about the unsuitability of the substrate detectable in the fungus, as presented in chapter 2, one day after the incorporation of the substrate, *naïve* foragers reject privet after 24 hours in the fungus garden. The presence of *experienced* foragers inside the nest has a significant influence on the decrease in acceptance of privet by *naïve* foragers.

Experimental series I was conducted to see if acceptance of privet by *naïve* foragers would decrease within eight hours of foraging with a majority of *experienced* foragers as well as an *experienced* fungus garden with the cue still detectable (day 1 after the incorporation of unsuitable privet). *Naïve* foragers were transferred with a majority of *experienced* foragers to an *experienced* fungus garden and acceptance of privet by *naïve* and *experienced* foragers was compared between *before transfer* and *after foraging* (Figure 5.4).

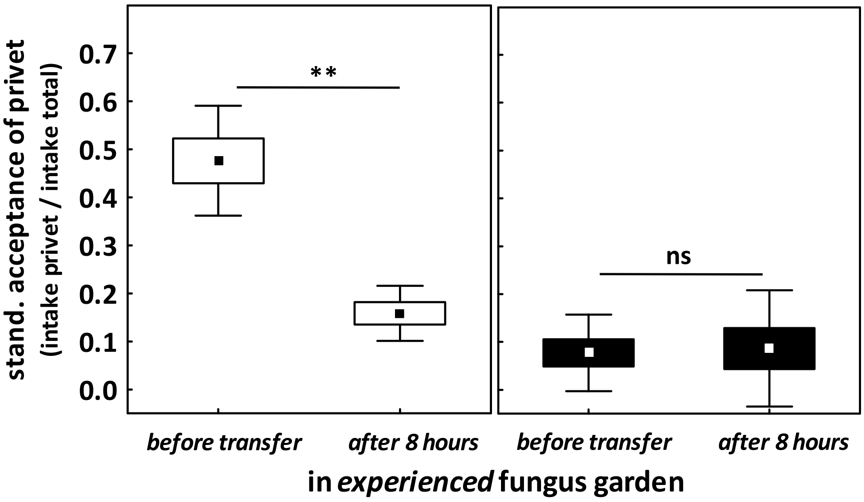

Figure 5.4: Acceptance of privet by *naive* and *experienced* foragers transferred to a fungus garden 1 day after treatment (series I). 200 *naive* (☐) and 500 *experienced* (■) foragers were transferred and acceptance of privet was tested *before transfer* and *after 8 hrs* of foraging. Data are mean ± SE with n = 6 subcolonies. Significance of paired t-tests is given as ns = non significant and **P < 0.01.

Accompanied by *experienced* foragers and with detectable cues in an *experienced* fungus garden, *naïve* foragers significantly lowered their acceptance of privet over an eight hour foraging period (p < 0.01, t = 5.12, df = 5). As foragers repeatedly visited the fungus garden throughout the day, two possible sources of information about substrate suitability were available: either from the *experienced* fungus garden directly during visits to the nest or from *experienced* foragers on the trail. Experienced foragers on the other hand showed no difference in the acceptance of privet *before transfer* and *after 8 hours* (p = 0.53, t = -0.67, df = 5). Even though they encountered *naïve* foragers on the trail and the harvested privet was suitable, *experienced* foragers still rejected the substrate after eight hours.

Experimental series II was aimed at the question if recent information about plant unsuitability through the fungus garden alone is enough to cause a significant decrease in acceptance of privet by *naïve* foragers. *Naïve* foragers were transferred alone to an *experienced* fungus garden one day after the incorporation of unsuitable privet (Figure 5.5).

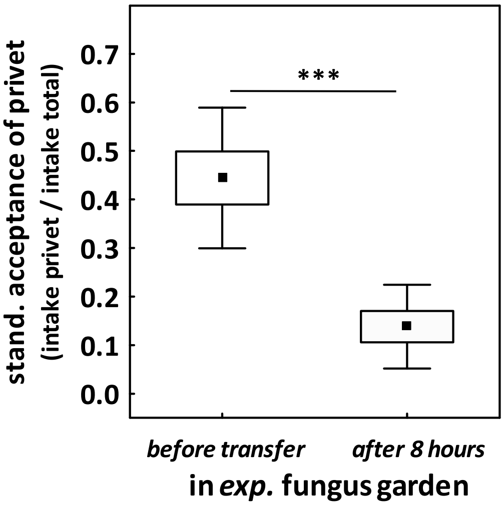

Figure 5.5: Acceptance of privet by *naïve* foragers transferred to an *experienced* fungus garden 1 day after treatment (series II). 700 *naïve* foragers were transferred and acceptance of privet was compared between *before transfer* and *after 8 hours* of foraging. Data are mean ± SE with n = 7. Significance of paired t-test comparing *naïve* foragers *before transfer* and *after 8 hours* is given as ***P < 0.001.

Even in the absence of *experienced* foragers, *naïve* foragers had significantly lowered their acceptance of privet after an eight hour foraging period ($p < 0.001$, $t = 8.26$, $df = 6$). Information about the unsuitability of privet inside the fungus garden was enough for *naïve* foragers to learn about privet's unsuitability.

To see if the presence or absence of *experienced* foragers had an influence on the magnitude of change in acceptance of privet by *naïve* foragers, decrease in acceptance of privet by *naïve* foragers was compared (Figure 5.6). There was no significant difference in the change of acceptance after 8 hours of foraging comparing the experimental series with and without *experienced* foragers present (series I & II) on day 1 after incorporation of unsuitable privet into the fungus garden ($p = 0.31$, $t = 1.07$, $df = 11$).

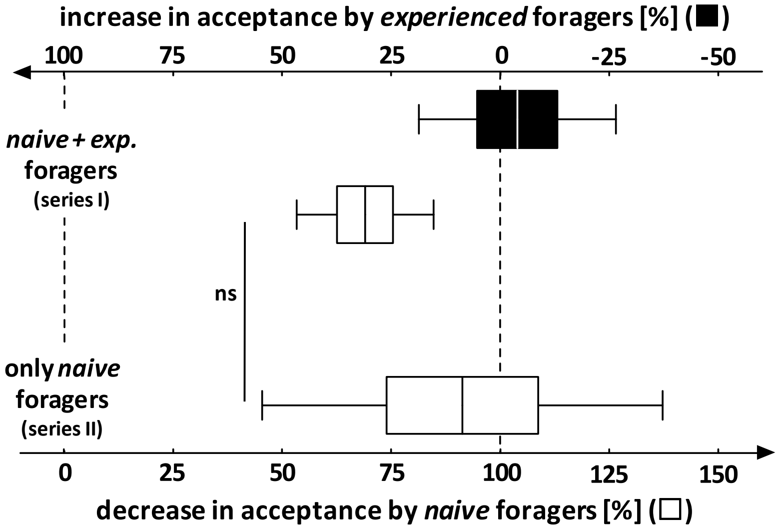

Figure 5.6: ***Naïve*** **foragers transferred to** ***experienced*** **fungus garden 1 day after treatment.** Change of acceptance over 8 hours of foraging with (series I) and without *experienced* foragers present (series II). Data are mean ± SE, with n = 6 (series I) and n = 7 (series II). 100 % = Difference in acceptance between paired *naïve* and *experienced* subcolonies *before transfer*. Series I: *naive before*: 47.60 ± 4.67, *exp. before*: 4.00 ± 1.91, 100 % = 43.60 ± 6.13. Series II: *naive before*: 44.45 ± 5.48, *exp. before*: 7.35 ± 3.08, 100 % = 37.10 ± 5.67. Significance of unpaired t-test is given as p = ns.

Influence of experienced foragers in absence of a cue in the fungus

On day 7 after the incorporation of unsuitable substrate, there is no cue in the fungus anymore, so information inside the fungus garden about the unsuitability of privet was only retrievable through *experienced* gardeners. In experimental series III, *naïve* foragers were transferred with a majority of *experienced* foragers into an *experienced* fungus garden on day 7 to see if *naïve* foragers could still learn about the unsuitability of privet even without the cue in the fungus (Figure 5.7). *Naïve* ants foraging together with a majority of *experienced* foragers accepted privet significantly less after eight hours even in the absence of a cue in the fungus garden ($p < 0.01$, $t = 6.21$, $df = 5$). *Experienced* foragers did not change their acceptance over time ($p = 0.92$, $t = 0.10$, $df = 5$).

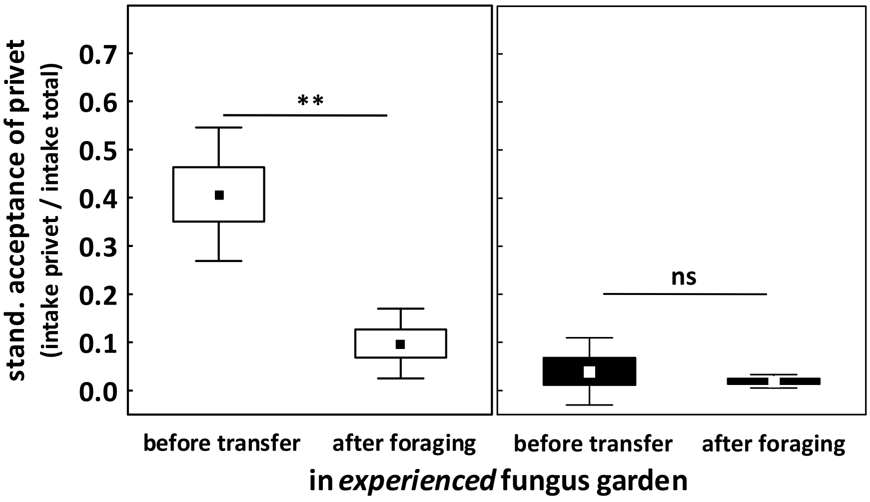

Figure 5.7: Acceptance of privet by *naive* (☐) and *experienced* (■) foragers transferred to a fungus garden 7 days after the treatment (series III). 200 *naive* and 500 *experienced* foragers were transferred and acceptance of privet was tested *before transfer* and *after 8 hours* of foraging. Data are mean ± SE with n = 6 subcolonies. Significance of paired t-tests is given as ns = non significant and **P < 0.01.

To see if *naïve* ants foraging on their own without the cue present in the fungus also lower their acceptance of privet within eight hours, experimental series IV was conducted. *Naïve* foragers were transferred alone to an *experienced* fungus garden on day 7 after the treatment with unsuitable privet (Figure 5.8).

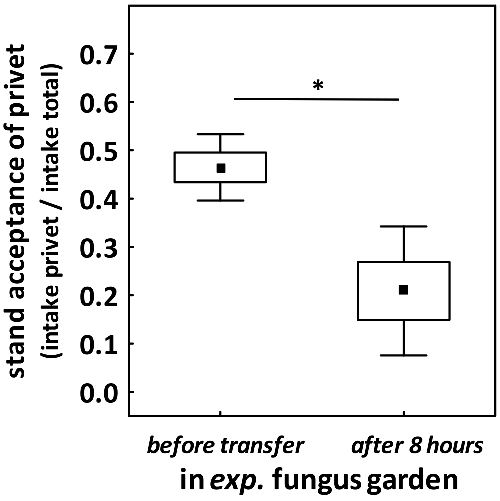

Figure 5.8: Acceptance of privet by *naïve* foragers transferred to an *experienced* fungus garden 7 days after the treatment (series IV). 700 *naïve* foragers were transferred and acceptance of privet was compared between *before transfer* and *after 8 hours* of foraging. Data are mean ± SE with n = 5. Significance of paired t-test is given as *P < 0.05.

On day 7, when information about the suitability of privet in the fungus garden was only retrievable through *experienced* gardeners, *naïve* ants lowered their acceptance of privet significantly over the course of 8 hours ($p < 0.05$, $t = 2.87$, $df = 4$).

To see if the presence or absence of experienced foragers had an influence on the magnitude of change in acceptance of privet by naïve foragers in the absence of the cue in the fungus, experimental series III and IV were compared (Figure 5.9).

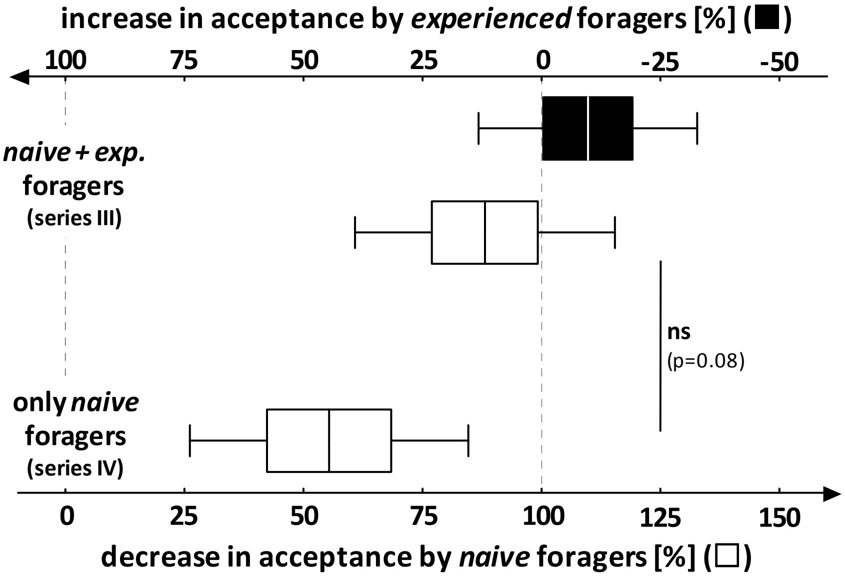

Figure 5.9: ***Naïve*** **foragers transferred to** ***experienced*** **fungus garden 7 days after treatment.** Change of acceptance after 8 hours of foraging with (series III) and without *experienced* foragers present (series IV). Data are mean ± SE, with n = 6 (series III) and n = 5 (series IV). 100 % = Differences in acceptance between *naïve* and *experienced* subcolonies *before transfer*. Series III: *naive before*: 40.71 ± 5.65, *exp. before*: 3.97 ± 2.86, 100 % = 36.74 ± 6.25. Series IV: *naive before*: 46.44 ± 3.06, *exp. before*: 1.30 ± 1.02, 100 % = 45.15 ± 3.17. Significance of unpaired t-test is given as p = ns.

There was no significant difference in the magnitude of change in acceptance of privet by *naïve* foragers due to the presence or absence of *experienced* foragers. Nonetheless there was a clear trend indicating that the presence of *experienced* foragers facilitates learning of *naïve* foragers about the unsuitability of privet (p = 0.08, t = -1.97, df = 9).

Influence of a cue in the fungus in absence of experienced foragers

Finally, the change in acceptance of privet by *naïve* foragers was compared between series II, (cue in fungus) and series IV (without cue in fungus). *Naïve* foragers in series II decreased their acceptance of privet about 91.32 ± 17.34 % (mean ± SE), whereas in series IV, in the absence of the cue in the fungus, *naïve* foragers only decreased their acceptance of privet about 55.39 ± 13.08 % (mean ± SE). Even though the difference between the two experimental series was not significant (p = 0.12, t = 1.68, df = 10), a clear trend could be seen suggesting that the presence of the cue in the fungus leads to a more pronounced change in acceptance.

Naïve foragers were able to learn about the unsuitability of privet under all conditions presented in the four experimental series within an eight hour foraging period. Presence or absence of *experienced* foragers and / or the cue in the fungus seemed to play a role in the level of rejection of the substrate by *naïve* foragers, but did not result in a significant effect at the end of the day.

As the presence of *experienced* foragers might have an immediate influence on the speed of change in acceptance of privet by *naïve* foragers, I had a closer look at the dynamics of rejection.

Dynamics of rejection - turning point in change of acceptance

To define the point in time were a change in acceptance of privet by *naïve* foragers occurred throughout the eight hour foraging period, results from the 12 preference tests were fitted by a nonlinear regression.

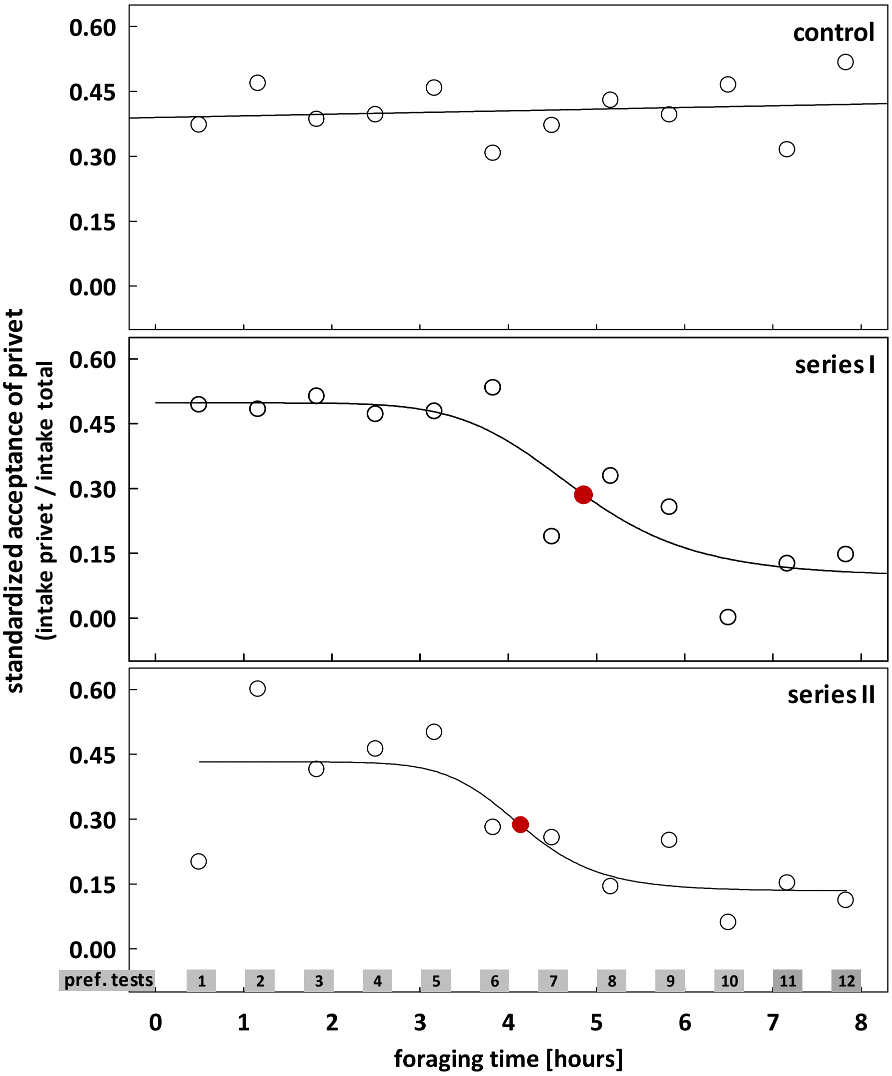

Figure 5.10: Examples for the acceptance of privet by *naïve* foragers throughout the eight hour foraging period. Control (*top*): *naïve* foragers transferred to a *naïve* fungus garden; **series I** (*center*): only *naïve* foragers transferred to an *experienced* fungus garden day 1 after treatment; **series II** (*bottom*): *naïve* together with *experienced* foragers transferred to an *experienced* fungus garden day 1 after treatment. Turning point in acceptance (●) was determined using a four parameter logistic curve (series I: $p < 0.01$, $r = 0.90$, slope: 7.05 and series II: $p < 0.05$, $r = 0.80$, slope: 9.40).

Fitting the data with a four parameter logistic curve (y = min + (max-min)/(1+(x/b)^a); min ≥ 0, max ≤ 1) between foraging time (x) and standardized acceptance of privet by *naïve* foragers (y), the turning point in acceptance (b) could be determined. Figure 5.10 shows the nonlinear decrease in acceptance over time and the turning point as the time of maximum decrease in acceptance in series I and II as well as acceptance of privet throughout the 8 hour foraging period in the control series for comparison. The four parameter logistic curve was determined for each trial in experimental series I - IV and only used if the model described more than 65 % of the data. Turning points in acceptance were then compared across experimental series.

On day 1 the cue about the unsuitability of the previously incorporated substrate is still present in the fungus. Acceptance of privet by *naïve* foragers transferred changed after around 4 hours of foraging (Figure 5.11).

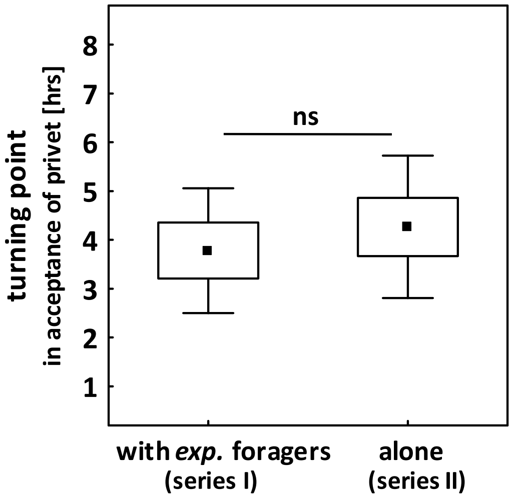

Figure 5.11: Turning point in acceptance of privet by *naïve* ants transferred to *experienced* fungus gardens on day 1 after treatment. Naïve ants foraged either with a majority of *experienced* ants (series I) or alone (series II). Data are mean ± SE with n = 5 (series I) and n = 6 (series II). Significance of unpaired t-test is given as ns = non significant.

There was no difference between the reaction time of *naïve* ants foraging by themselves and *naïve* ants foraging together with a majority of *experienced* ants (p = 0.58, t = -0.58, df = 9). The presence of a majority of *experienced* ants on the trail did not lead to a significantly earlier change in acceptance of privet by *naïve* foragers on day 1. On day 7 however, the cue in the fungus garden was not present anymore, and only the *experienced* gardeners could convey the information about the substrate quality in the presence of the substrate in question. In the presence of *experienced* foragers, the turning point in acceptance of privet by *naïve* foragers occurred 3.70 ± 0.57 hours after the start of the foraging period (Figure 5.12).

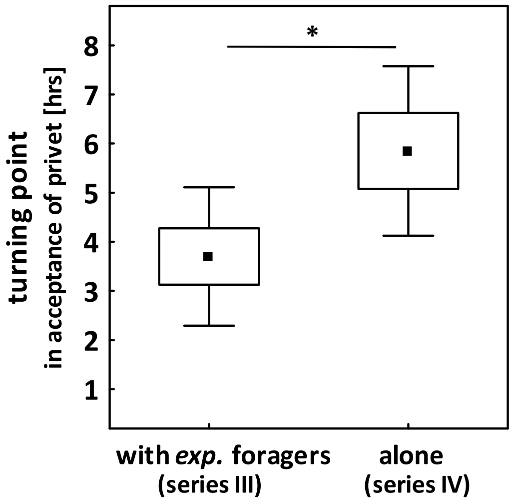

Figure 5.12: Turning point in acceptance of privet by *naïve* ants transferred to *experienced* fungus gardens on day 7 after treatment. Naïve ants foraged either with a majority of *experienced* ants (series III) or alone (series IV). Data are mean ± SE with n = 6 (series III) and n = 5 (series IV). Significance of unpaired t-test is given as *P < 0.05.

The acceptance of privet by *naïve* ants foraging alone changed significantly later, after 5.85 ± 0.77 hours. In the absence of the cue in the experienced fungus (day 7), the presence of *experienced* foragers led to an earlier change in acceptance of privet by *naïve* foragers.

Influence of naïve-experienced ratio on the trail - detailed

Ratio of *experienced* and *naïve* foragers transferred was always 500 *experienced* versus 200 *naïve* foragers i.e. about 70 % of all foragers in the subcolony were *experienced.* Traffic counts conducted during each of the 12 preference tests were used to calculate the mean percentage of active foragers throughout the foraging period for each trial. In series II, percentages ranged across trials from 64.8 % to 94.6 % of *experienced* foragers on the trail. To investigate the influence of *experienced* foragers their mean activity throughout the eight hours of foraging was correlated with the level of acceptance of privet by *naïve* foragers after those eight hours (Figure 5.13).

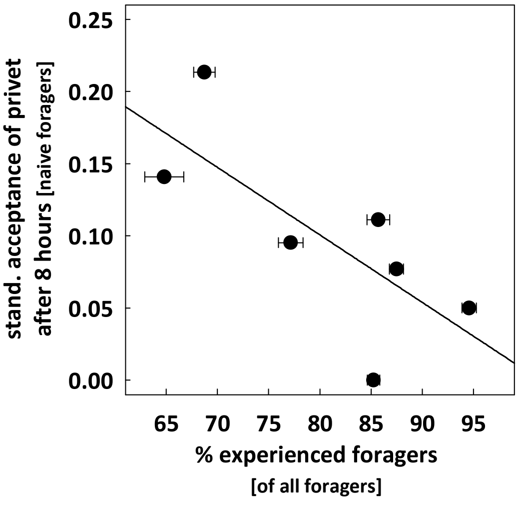

Fig. 5.13: Standardized acceptance of privet by *naive* foragers after 8 hours of foraging in relation to the percentage of *experienced* foragers on the trail throughout the foraging period. Data are mean ± SE with linear regression; r = 0.74; slope: -0.005.

There is a trend that a higher percentage of *experienced* foragers on the trail throughout the eight hour foraging might lead to a lower acceptance of privet by *naïve* foragers at the end ($p = 0.06$, $r^2 = 0.55$).

As only certain *experienced* foragers encountered along the way might influence the response of *naïve* foragers, counts of *experienced* foragers were split into laden and unladen foragers on their way back to the nest and foragers coming from the nest travelling towards the food source (Figure 5.14).

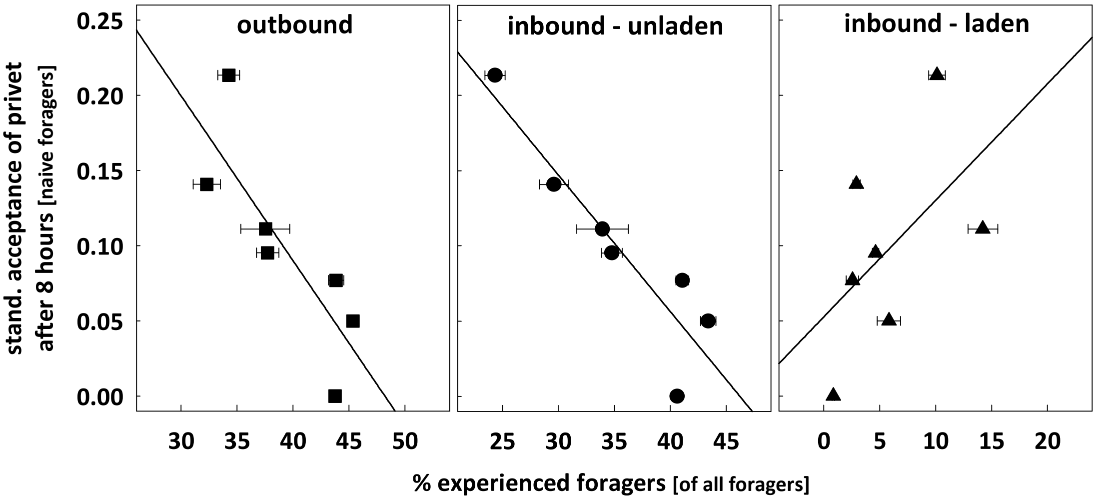

Fig. 5.14: Standardized acceptance of privet by *naive* foragers after 8 hours of foraging in relation to the percentage of *experienced* foragers on the trail throughout the foraging period. Data are mean ± SE with linear regressions. Outbound (*left*): r = 0.83; slope: -0.01; inbound - unladen (*center*): r = 0.92; slope: -0.01; inbound - laden (right): r = 0.54; slope: 0.01.

Acceptance of privet by *naïve* foragers correlated strongly with the percentage of *experienced* foragers travelling outbound to the food source ($p < 0.05$, $r^2 = 0.68$). Looking at the percentage of unladen *experienced* foragers travelling inbound back to the nest, there was also a high correlation with the acceptance of privet by *naïve* foragers after eight hours ($p < 0.01$, $r^2 = 0.84$). No correlation could be found though between the percentage of laden *experienced* foragers (almost exclusively laden with bramble) travelling inbound and the acceptance of privet by *naïve* foragers after foraging ($p = 0.21$, $r^2 = 0.29$).

Discussion

Foragers decide on plant material for their symbiotic fungus out in the field based on innate tendencies and experience. Some plants species are evaluated by foragers as suitable but prove to be unsuitable for the symbiotic fungus after their incorporation. Due to a feed-back mechanism from the fungus, foragers then reject the unsuitable substrate. Information acquisition about the unsuitability of a substrate already harvested is of great importance for the colony in order to minimize the intake of said substrate and prevent their symbiotic fungus from further harm.

Influence of experienced foragers - in the presence of a cue in the fungus

If effects of the unsuitable substrate on the fungus as well as the substrate's identity are still identifiable inside the fungus chambers, i.e. a cue is present, *naïve* foragers' acceptance of the substrate decreases significantly within eight hours of foraging. This shows that visits to the garden between foraging bouts are sufficient to learn about the previous unsuitability during one foraging day. Preliminary observations suggest that all foragers will have visited the nest at least once within 2-3 hours of foraging. The strongest decrease in acceptance of privet by *naïve* foragers was reached within four hours of foraging suggesting that it probably takes more than one visit to learn about the previous unsuitability of privet and that a majority of *naïve* foragers had already learned about it at that time (series II).

Interestingly, the presence of *experienced* foragers on the trail seemed to have no influence on the speed of rejection of privet by *naïve* foragers. In the presence of the fungal cue, *naïve* foragers changed their acceptance of privet within 4 hours of foraging irrespective of the presence or absence of *experienced* foragers on the trail. This could either be due to the effect of the unsuitable substrate on the fungus being so strong that the presence of *experienced* foragers is irrelevant in speeding up the process of initial information transfer or the presence of *experienced* foragers generally has no negative influence on the acceptance of the unsuitable substrate by *naïve* foragers.

Influence of experienced foragers - in absence of a cue in the fungus

I could show in previous experiments (chapter 2) that in the absence of a cue from the fungus, the presence of a majority of *experienced* foragers inside the nest had a significant influence on the decrease of acceptance of privet by *naive* foragers 24 hours later. This shows that under certain conditions, the presence of *experienced* foragers indeed influences acceptance of substrate by *naïve* foragers. Therefore it seems likely that through the presence of information about the unsuitability of substrate in the fungus, the potential effect of the *experienced* foragers' presence might be superimposed.

In absence of a cue in the fungus garden, *naïve* foragers also significantly lowered their acceptance of privet after eight hours of foraging. They learned about the previous unsuitability of privet even though the only source of information was the presence of *experienced* gardeners. The additional presence of *experienced* foragers tended to reduce the level of acceptance of privet by *naïve* foragers after eight hours, though not significantly so. A close analysis of the dynamics of rejection over these eight hours showed that the presence of *experienced* foragers had significant influence on the turning point in acceptance of privet by *naïve* foragers though. The strongest change in acceptance of privet by *naïve* foragers in absence of *experienced* foragers occurred after around 6 hours of foraging; if *experienced* foragers were present, this change in acceptance already occurred after 4 hours. This shows that *naïve* foragers are significantly faster informed about the previous unsuitability of privet in the presence of *experienced* foragers.

Naïve foragers show the same speed in decreasing their acceptance of privet regardless if information is provided directly through a cue in the fungus (1-2 days after initial incorporation of substrate into the fungus) or several days later when the effects in the fungus are not detectable anymore indirectly through *experienced* gardeners and *experienced* foragers only.

A fast colony response (colony rejection) is possible at a point in time when information about its unsuitability is only present through *experienced* gardeners and foragers towards a substrate that has previously been experienced as unsuitable. This might partly explain the occurrence of rejection lasting up to 3-4 months after the initial acceptance of the substrate by adult colonies of *Atta colombica* in the field (Saverschek et al. 2010) as a majority of *experienced* foragers and gardeners seems to be sufficient to reinforce rejection of the substrate on the trail at a point when no cue is present in the fungus anymore.

Influence of naïve-experienced ratio on the trail - detailed

Presence of *experienced* foragers only had a significant influence on *naïve* foragers in the absence of a cue in the fungus. In *Acromyrmex ambiguus*, average walking speed of laden ants is 1.0 m/min regardless of the suitability of the substrate they carry (chapter 6). These results from the lab are congruent to travelling speed of *Atta* in the field (1.0-1.3 m/min Lewis et al. 1974; Cherrett 1989). In the experimental set-up with the food source placed 4 m away from the nest, foragers could therefore complete a round trip within 8 minutes not taking into account the time it took foragers to choose and pick up a leaf disk. Of all laden foragers reaching the nest, 86 % enter the nest with their load. This suggests that *naïve* foragers had repeatedly visited the fungus garden before the turning point in acceptance occurred. As plant preferences of foragers are not regulated by simple yes-no answers a single nest visit presumably only decreases the likeliness of *naïve* foragers to pick up privet leaf disks. Nest visits might therefore have a significant effect on plant preferences of *naïve* foragers if they occur frequently due to a short trail length, but have probably only some effect in the field, only slowing down the intake of the unsuitable material, without stopping it on the same day because foragers in the field enter the nest less frequently.

With trails lengths up to 25-30 m in *A. ambiguus* (Bonetto 1959), a round trip in the field can last about one hour not taking into account the time it takes for the forager to choose, cut and pick up a leaf fragment and potential disturbances on the trail like high traffic volume or physical barriers. Even though this suggests that visits to the nest are rare throughout a single foraging period, even a single nest visit might be enough for naïves workers to learn about the negative effects the substrate has previously caused in the fungus. For example, workers of the grass-cutting ant *Acromyrmex lundi* can be conditioned to odors of a burden carried by a single scout encountered during recruitment (Roces 1990) and an unladen ant of *Atta cephalotes* that undergoes a head-on encounter with a laden ant increases its probability of reaching food from 2% to 40% (Farji-Brener et al. 2010).

In the field, where trails are longer and visits to the nest are less frequent (Cherrett 1983), the presence of *experienced* foragers on the trail might be of bigger importance than on short trails in the laboratory.

My data show that there is a correlation between level of acceptance of privet by *naïve* foragers after the eight hour foraging period and percentages of *experienced* workers on the trail throughout these eight hours. It seems like the presence of a higher percentage of *experienced* foragers leads to lower acceptance of privet by *naïve* foragers. According to Howard et al. (1996) this could be due to the conditioning of *naïve* foragers travelling towards the food source through loads carried by the majority of foragers they encounter along the way. *Experienced* foragers carried almost exclusively blackberry, but the percentage of laden *experienced* foragers back to the nest has no influence on the plant preferences of *naïve* foragers. As laden *experienced* foragers make up 80 % of all laden ants returning to the nest, according to Howard the load predominantly encountered on the way to the food source should be the one preferred. Results from the control colony in which acceptance of privet was monitored over the course of eight hours could not support this. Privet intake even slightly increased throughout these eight hours though the majority on the trail harvested blackberry. Results found in the field in *Atta colombica* (Howard et al. 1996) are better explained through habitat effects and the influence of early experience on substrate preferences (*Acromyrmex landolti fracticornis,* Fowler 1982*; Atta colombica*, Saverschek et al. 2010).

A considerably high percentage of *experienced* foragers on the trail (25-45 %) return to the nest without a load. This is congruent to data collected in other species (13-75%, Hodgson 1955; Cherrett 1972; Lugo et al. 1973; Lewis et al. 1974b) and has been party explained with some being involved in trail-clearing (Daguerre 1945) transporting plant sap (Stradling 1978), or engaged in the reinforcement of the chemical trail (Jaffe & Howse 1979). Interestingly, my results show a high correlation of the percentage of unladen workers with the decrease in acceptance of privet by *naïve* ants. This holds true for all unladen *experienced* foragers, irrespective if they return to the nest from the food source or if they are outbound ants. In recent years, studies on traffic flow had a closer look at head-on encounter rates and their possible role in the transfer of information (Gordon & Mehdiabadi 1999) Burd & Aranwela 2003; Farji-Brener et al. 2010). In *Atta cephalotes*, outbound ants experienced significantly higher encounter rates than unladen but returning ants. The difference cannot be due to load carriage, and it has been speculated that outbound ants are actively seeking encounters with incoming ants (particularly laden ants) for information acquisition (Burd & Aranwela 2003). When it comes to the transfer of information about plants' unsuitability on the trail, unladen *experienced* foragers might contact laden workers not necessarily to receive information, but to actively transmit information about the suitability of the load they carry. I took a closer look at encounters of laden *naïve* foragers on the way to the nest in chapter 6.

Conclusions

Plant species' suitability for the fungal symbiont differs among individuals of the same plant species and changes seasonally (Fowler & Stiles 1980). A suitable substrate today might be unsuitable tomorrow and vice versa. In the absence of effects of the substrate on the fungus, *naïve* foragers decrease their acceptance of a substrate due to the presence of foragers and gardeners having experienced said substrate as unsuitable. *Naïve* foragers do not evaluate the suitability of the substrate themselves, but after recognition rely on information provided by others leading to the rejection of a potentially suitable substrate. This inertia in reacting to the changed suitability of a substrate known to parts of the colony as unsuitable is the side effect of a system in which it is highly important to prevent the intake of unsuitable substrate. Change in acceptance can occur much faster if assessment of plant suitability can be indirect as well as direct (Pernal & Currie 2001).

The turning point in acceptance of privet by *naïve* foragers occurs earlier, if *experienced* foragers are present additionally to *experienced* gardeners in the nest. Several sources of information lead to a faster distribution as chances of encountering this information increases which means that more individuals are informed faster. The fungal cue seems to be the strongest influence, as in its presence, the presence of experienced foragers has no significant influence. The relevant influence of each source of information might be dependent upon the frequency with which foragers return to the nest throughout the day and the ratio of *naïve* to *experienced* foragers on the trail.

CHAPTER 6

INTERACTIONS BETWEEN LADEN FORAGERS AND THEIR NESTMATES ON THE WAY BACK TO THE NEST: THE INFLUENCE OF SUBSTRATE SUITABILITY

Abstract

In leaf-cutting ants, information transfer about plant suitability during foraging is important and has up to know mostly been investigated in the initial phase of recruiting to a newly discovered food source. Due to the unique feed-back system via the symbiotic fungus and the resulting time delay of information availability, it is also still important when food sources are already fully established. Depending on whether or not an individual has already learned about a plant's negative effects on the fungus, foragers with different experiential backgrounds regarding the plant species travel together on the same trail. Naïve foragers decrease their acceptance of substrates in the presence of foragers that have negative previous experience with it. In this study, laden individuals were observed coming from a fully established food source and differences in the behavior and interactions with nestmates due to different experiential background and the perceived suitability of the carried load were recorded. Head-on encounter rates did not differ, whether nestmates perceived the load as suitable or unsuitable, but active contacts occurred significantly more often if nestmates had previously encountered the carried substrate as unsuitable. These contacts were initiated by the nestmates and consisted of either antennating the laden individuals' body or load or biting into the carried leaf disk. No difference in the occurrence of mutual antennation could be found between laden recruits travelling among naïve or experienced foragers. Even though recruits carrying a load perceived as unsuitable by their nestmates were contacted significantly more often, these interactions did not result in a longer travel time. There was also no difference in the percentage of laden recruits entering the nest with their load due to the received attention by nestmates. Increased number of contacts initiated by nestmates on the trail might activate foragers to re-evaluate their load again and look for information inside the fungus garden. Once inside the nest though, foragers stayed twice as long if the substrate was known as suitable by the subcolony than when the substrate was known as unsuitable, maybe due to the fact that if the substrate was previously unsuitable, detrimental effects on the fungus were easily detectable. Laden foragers on their way back to the nest experience significantly different behavior from nestmates due to the nestmates' experience or lack thereof with the carried substrate. It seems likely that foragers actively influence each other on the trail in their substrate preferences through contacts on the trail thus accelerating the colony wide rejection of substrates proven to be unsuitable for their symbiotic fungus.

Introduction

In social insects, evaluation of substrate suitability takes place on an individual level and acceptance gets validated or dismissed through social interactions among workers. In leaf-cutting ants, assessment of host plant suitability is an elaborate process consisting of several steps as workers harvest food not for themselves, but as substrate to culture their mutualistic fungus. Some substrates are evaluated by workers as suitable, but are nonetheless harmful to the fungus due to components undetectable by the ants. If this is the case, foragers afterwards reject the substrate that has proven to be unsuitable for the fungus (chapter 2 & 5). This takes considerable time, between several hours and 2-3 days for complete rejection of the substrate on all trails of a colony (Herz et al. 2008; Saverschek et al. 2010).

Leaf-cutting ant colonies usually harvest several plant species simultaneously on different trails and a considerable percentage of foragers changes trails every day (15-45 %, Porter and Bowers 1982, Wagner 2004). Foragers of different experiential background are therefore present within one colony possibly foraging together on the same trail. Depending on whether or not foragers already learned about a substrate's negative effect on the fungus, it could be evaluated as suitable or unsuitable by different foragers within the same colony. Experiments have shown that the presence of experienced foragers, individuals with previous experience regarding the unsuitability of a substrate, facilitates the decrease in acceptance of that substrate by naïve foragers. In the absence of the harmful effects on the fungus, experienced gardeners as well as foragers can communicate their previous experience with the unsuitability of the plant species to naïve foragers inside the nest but it only takes place in the presence of the substrate in question (chapter 2).

Results from previous studies (chapter 5) indicate that foragers carrying a substrate they consider suitable might be influenced in their foraging decisions through unladen foragers that experienced said substrate as unsuitable in the past. As foragers spend a considerable amount of time every day on the trail (Shepherd 1982), it seems conceivable that information about the unsuitability of a substrate is communicated there, as well as in the nest.

Interactions on the trail among foragers are already known during recruitment (Roces 1990; Howard et al. 1996) inadvertently leading either to the ceasing of a food source if substrate seems to be unsuitable to a majority of foragers or on the contrary, to an established foraging trail if the substrate appears to be suitable. Due to the time delay in rejection caused by the fungus' reaction to the unsuitable substrate (10 hours in small laboratory colonies, Herz et al.

2008), foraging at this particular food source might have been already fully established in the meantime.

Factors influencing foragers on the trail already known are passive. Mostly, foragers are exposed to inadvertent cues through the mere presence of foragers carrying certain loads (Howard et al. 1996) and positive, i.e. increasing the likeliness that foragers choose the substrate they have encountered on the trail carried by a nestmate (Roces 1990, 1994; Farji-Brener et al. 2010).

In this study, the influence of differing experiential backgrounds of foragers, regarding the suitability of harvested substrates, on the behavior of individuals on the trail were examined. A familiar, suitable plant species was presented and laden foragers returning from the established food source were then individually transferred to another subcolony, in which their nestmates considered their load as either suitable or unsuitable depending on the experimental series. Encounters between the observed laden individual and its nestmates were recorded to see if and how foragers might interact differently due to the perceived suitability of an individual’s load. These differences in behavior could indicate the influence of nestmates on the trail on substrate choice of individuals.

Additionally, the time recruits spend on the trail was measured to see if reduced travel speed might be in indirect factor in the overall decrease of acceptance of unsuitable substrate. Time spend inside the nest was might vary depending on the information obtainable and was therefore recorded as well.

Methods

In two experimental series, recruits transferred to another subcolony either carried a privet leaf disk known as either suitable (*control*) or unsuitable (*treatment*) to the other foragers on the trail.

Subcolonies

Experiments were conducted with subcolonies obtained from 5 large lab colonies of *Acromyrmex ambiguus*. The colonies were collected in Uruguay in 2002 and maintained in the laboratory at 25°C and under a LD cycle of 12:12 h. Artificial nests consisted of three transparent Plexiglas®-boxes connected by short PVC-tubes (10 cm, Ø 3 cm). The center box (19 x 8.5 x 8.5 cm) served as the fungus chamber and the two other boxes (19 x 19 x 8.5 cm) as feeding-box and refuse-box respectively. The bottom of the fungus-box was covered with moistened expanded clay pebbles to keep the humidity high and prevent desiccation of the fungus. The lids of the refuse- and feeding-boxes had three holes each (Ø 3 cm), covered with a fine metallic mesh allowing air circulation to create a different, dryer microclimate from the one inside the fungus-box, an *inside* and *outside* for the subcolony.

About 1000 ml of fungus garden (fungus and gardening workers) were taken from the mother colony and placed in the artificial nest together with approximately 1000 foragers. Subcolonies were established at least 4 days prior to the start of the experiment to ensure well established fungus gardens and active foraging behavior. The subcolonies received fresh blackberry leaves (*Rubus fructicosus*) and water every day and honey water every other day.

Experimental set-up

Two subcolonies were connected to identical set-ups with foraging trails running parallel and facing the same direction. The feeding-boxes of the subcolonies were disconnected and replaced with a PVC-tube each leading directly from the fungus chamber to the top of the entry platform (Figure 6.1). From this platform, a 5 cm wide and 3.5 m long wooden bridge (*main trail*) lead to another box (19 x 19 x 8.5 cm) used as experimental feeding arena. One subcolony served as source subcolony, the other as experimental subcolony.

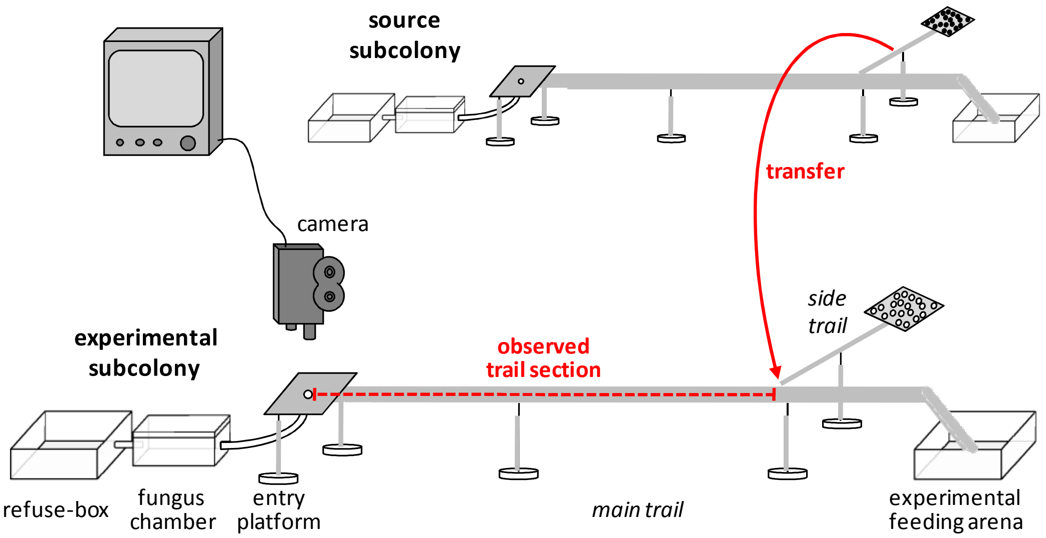

Figure 6.1: Experimental set-up. A wooden bridge connected the entry platform with the experimental feeding arena 3.5 m away. The *side trail* from which individuals were transferred to the experimental subcolony was placed 50 cm away from the experimental feeding arena. A camera was installed above the entrance hole of the experimental subcolony.

A *side trail* consisting of a platform (10 x 10 cm) and two parallel short wooden bridges (10 x 0.5 cm) was connected to the *main trail* 35 cm away from the experimental feeding arena. Above the entrance hole of the experimental subcolony, a camera was installed. Subcolonies foraged along the main trail for at least 2 days before the start of the experiment.

Experimental procedure

Laden recruits on their way back from the food source in the source subcolony, were individually transferred via the flexible side trail to the main trail of another subcolony (experimental subcolony). Interactions on the way back to the nest along the main trail of the experimental subcolony were recorded.

Two experimental series were conducted with privet (*Ligustrum vulgare*) as the test plant species and blackberry as the familiar, suitable reference plant species and main food source. In both experimental series, source subcolonies were *naïve*, i.e. privet was known as suitable. In the *control* series, experimental subcolonies were *naïve* as well, i.e. privet was known as suitable whereas in the *treatment* series, privet was known as unsuitable to the *experienced* experimental subcolonies.

Treatment of experimental subcolonies: to create different experiential backgrounds, subcolonies were exposed to one of two treatments. In the *control* series, they received 110 untreated, suitable leaf disks of privet (from now on: *naïve* subcolonies). In the *treatment* series, they received 110 leaf disks of treated, unsuitable privet (from now on: *experienced* subcolonies). The treated leaf disks were infiltrated with CHX (see chapter 1).

To guarantee well established foraging, all subcolonies foraged along the main trail for at least 2 days previous to the experiment. They only received fresh blackberry leaves in the experimental feeding arena before and during experiments. On the day of the experiment, the colony was given time (between 20-30 min) to establish the foraging process, until a constant number of laden foragers returned from the feeding arena at the end of the *main trail* (blackberry leaves) as well as the platform at the end of the *side trail*. The source subcolony received privet leaf disks on the *side trail*, the experimental subcolony received blackberry leaf disks. Leaf disks (Ø 6 mm) were always punched out freshly throughout the experiments.

In the source subcolony, a forager picking up a leaf disk on the platform was marked individually with a small dot of paint (Edding® 750) on the thorax. The paint was carefully applied with the tip of a bent paper clip without disturbing the forager. As the *side trail* consisted of two parallel narrow wooden bridges, one of the two could be briefly removed to transfer a laden forager to the experimental subcolony without interrupting the foraging flow to and from the platform of the source subcolony. When the marked forager had left the platform and entered the *side trail* with its leaf disk, the bridge was carefully moved to the experimental subcolony. Once the forager reached the end of the bridge, it entered the *main trail* of the experimental subcolony at the intersection of *side trail* and *main trail*. Observation started when the forager entered the *main trail* and ended once the forager reached the nest entrance.

Observed interactions:

Head-on encounters: all encounters of the individual with other nestmates whether they appeared to be mere collisions with nestmates or ***active contacts*** involving interactions with nestmates or by nestmates towards the recruit.

Recording active contacts, I distinguished between contacts in which both individuals were active and contacts in which the observed recruit was approached by nestmates.

Mutual antennation: observed laden recruit mutually antennating with nestmates head-to-head on its way to the nest.

Contacts initiated by nestmates:

Antennation of leaf or recruit by nestmates: nestmates drumming with their antennae on any body part or the leaf disk the observed individual is carrying.

Received bites into leaf by nestmates: nestmates biting into the leaf disk carried by the observed recruit.

It was also recorded how long it took the recruit to return along the 315 cm long trail to the nest and if it entered the nest. To calculate the duration of stay inside the nest, time of entry and exit was recorded with a camera placed above the entrance hole of the nest.

Data analysis

Observer 2.0®, a DOS-based program for observational data collection (Noldus Information Technology, Wageningen, Netherlands) was used to record observations along the trail.

In the *control* series, 178 foragers were tested from 7 different subcolonies and in the *treatment* series, 158 foragers were tested from 6 different subcolonies. Data was pooled for each series. The occurrence and frequency of all behaviors was recorded. Differences between experimental series were tested with chi^2-tests, Mann-Whitney-U-tests and unpaired t-tests depending on the data.

Results

In both experimental series (*control* and *treatment*) tested ants could be put into two behavioral categories. Of all tested ants, 94 % carried their leaf fragment all the way back to the nest. The other 6 % dropped or transferred their leaf fragment close to the intersection where they entered the *main trail* coming from the food source on the platform. As the focus of this study lay on interactions of recruits on the way back to the nest, only ants carrying their fragment all the way back to the nest were included in the analysis.

Travel time and head-on encounters on trail

Foragers travelled 315 cm from the intersection where they entered the *main trail* from the *side* trail back to the nest entrance. Time spend on the trail was compared between recruits carrying a load known as suitable (*control*) or unsuitable (*treatment*) to its nestmates (Figure 6.2).

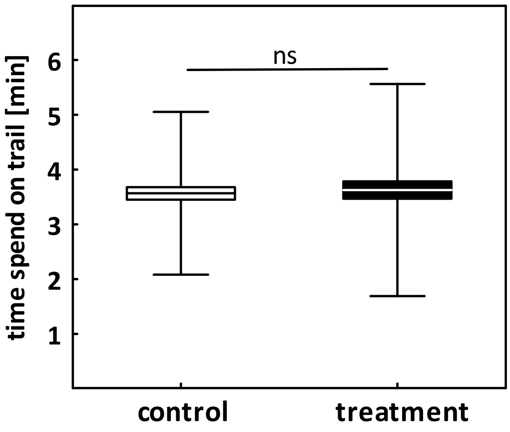

Figure 6.2: Time spent on trail [min] in *control* (☐) and *treatment* (■). Travel time measured from intersection to the nest entrance. N (*con/treat*): 167/149; p = 0.75, t = -0.32, df = 314. Data is shown as mean ± SE and significance of unpaired t-tests is given as ns = non significant.

In both experimental series, recruits travelled on average 3.6 minutes on the trail until they reached the nest. Throughout the trip, recruits had on average 60 head-on encounters with nestmates along the way in both experimental series (p = 0.08, t = 1.78, df = 314, n (*con/treat*): 167/149). Most of them were mere collisions without visual interaction, whereas others were active contacts between recruit and nestmate. With recruits carrying a leaf disk known to its nestmates as suitable, 6.5 ± 0.5 % of all head-on encounters were active (Figure 6.3), whereas recruits carrying a leaf disk known as unsuitable to the rest of the subcolony had a significantly higher percentage of active contacts (9.7 ± 0.7 %).

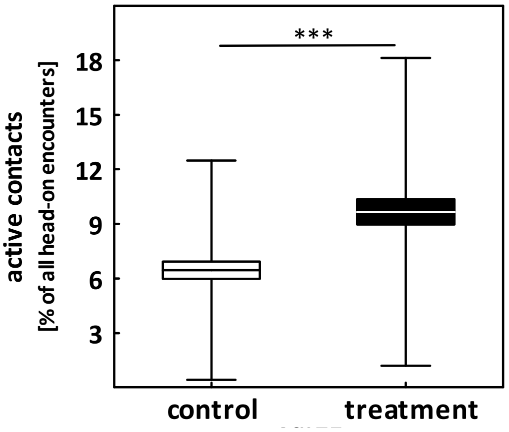

Figure 6.3: Active contacts as percentage of all head-on encounters of recruits in *control* (☐) and *treatment* (■). N (*con/treat*): 167/149; $p < 0.001$, t = -3.19, df = 314. Data is shown as mean ± SE and significance of unpaired t-tests is given as ***$p < 0.001$.

Mutual antennation

In both experimental series, slightly more than 50 % of all recruits mutually antennated with nestmates on their way to the nest (Figure 6.4). There was neither a significant difference in the percentage of ants antennating nor in the frequency. Recruits antennated twice on average with nestmates throughout the trip.

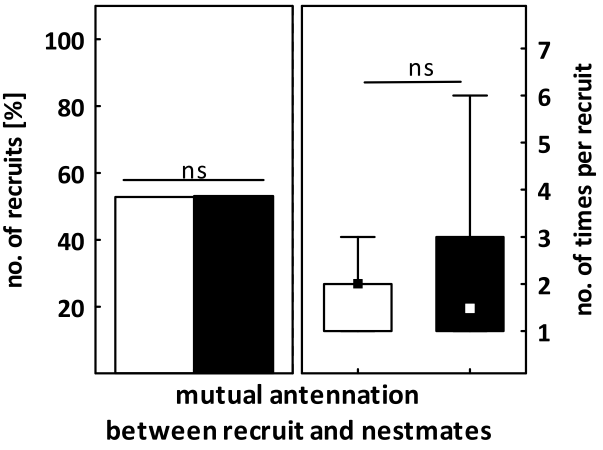

Figure 6.4: Occurrence of mutual antennation in *control* (☐) and *treatment* (■). *Left*: Percentage of recruits mutually antennating with other nestmates on the way to the nest. N (*con/treat*): n = 167/149, p = 0.95, chi^2 = 0.00. Significance of chi^2-test is given as ns = non significant. *Right*: Number of times behavior occurred per recruit. N (*con/treat*): n = 94/84, p = 0.88, U = 3895.00, Z = 0.15. Data is shown as median, quartile range and range. Significance of MWU-tests is given as ns = non significant.

Contacts initiated by nestmates

Nestmates antennated the leaf fragment carried by the recruit as well as the recruit itself and sometimes bit into the leaf fragment. Overall, more than 70 % of all recruits received antennation on load or body and leaf bites along the way in both experimental series (Figure 6.5). The frequency was significantly higher in the treatment series, with recruits being contacted by nestmates on average 6.3 ± 0.6 (mean ± SE) times per individual, whereas recruits in the control series were only contacted 3.9 ± 0.4 (mean ± SE) times.

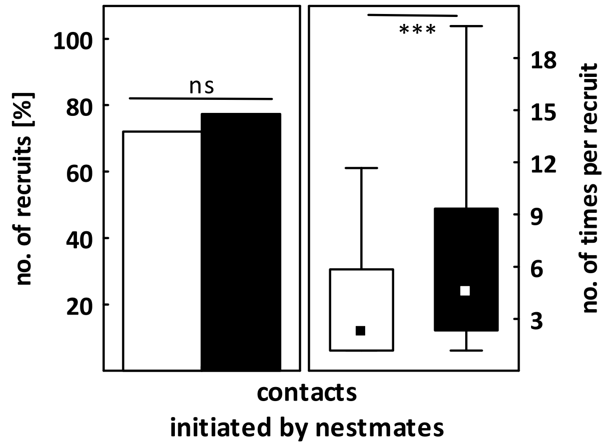

Figure 6.5: Recruits being contacted by nestmates in *control* (☐) and *treatment* (■). *Left*: Percentage of recruits being contacted by nestmates along the way. N (*con/treat*): n = 167/149, p = 0.27, chi^2 = 1.22. Significance of chi^2-test is given as ns = non significant. *Right*: Number of times behavior occurred per recruit. N (*con/treat*): n = 121/116, p < 0.001, U = 5211.50, Z = -3.42. Data is shown as median, quartile range and range. Significance of MWU-tests is given as ***p < 0.001.

Antennation of leaf or recruit by nestmates: Antennation of leaf or carrier was observed in both experimental series (Figure 6.6). There was no significant difference between *control* and *treatment* in the percentage of recruits receiving antennation with more than 70 % of all recruits in both experimental series. Recruits carrying a leaf disk known as suitable received significantly less antennation per individual (3.3 ± 0.3, mean ± SE) than recruits carrying a leaf disks known as unsuitable to the nestmates and receiving 5.5 ± 0.5 (mean ± SE) antennations on leaf or body on their way to the nest.

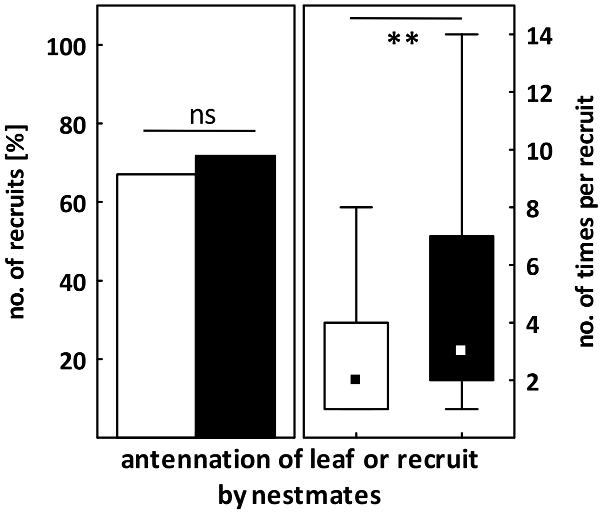

Figure 6.6: Antennation of leaf or recruit by nestmates in *control* (☐) and *treatment* (■). *Left*: Percentage of recruits being antennated on leaf or body by nestmates. N (*con/treat*): n = 167/149, p = 0.36, chi^2 = 0.83. Significance of chi^2-test is given as ns = non significant. *Right*: Number of times behavior occurred per recruit. N (*con/treat*): n = 121/107, p < 0.01, U = 4663.50, Z = -2.83. Data is shown as median, quartile range and range. Significance of MWU-tests is given as **p < 0.01.

Received bites into leaf by nestmates: Of all recruits travelling to the nest with their load, percentage of individuals receiving bites in the *control* series (33.7 %) was significantly lower than in the *treatment* series (45.6 %; Figure 6.7). Recruits carrying a leaf disk known to the nestmates as unsuitable also received significantly more leaf bites (3.3 ± 0.3, mean ± SE) than an individual carrying a leaf disk known as suitable (2.0 ± 0.3, mean ± SE).

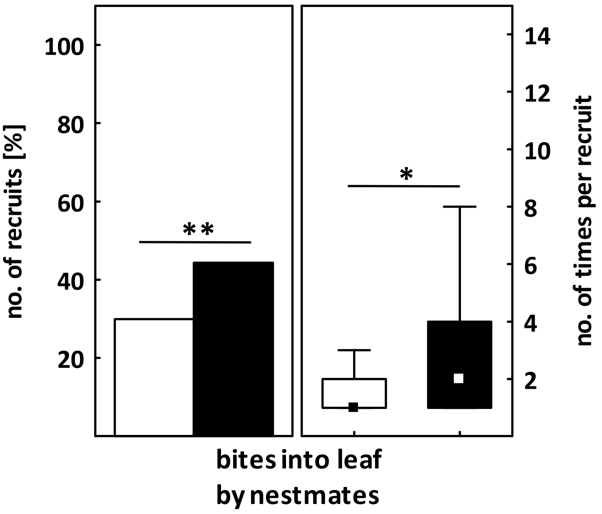

Figure 6.7: Bites into leaf by nestmates in *control* (☐) and *treatment* (■). *Left*: Percentage of recruits receiving leaf bites by nestmates. N (*con/treat*): n = 167/149, p < 0.01, chi^2 = 6.98. Significance of chi^2-test is given as **p < 0.01. *Right*: Number of times behavior occurred per recruit. N (*con/treat*): n = 50/66, p = 0.04, U = 1273.00, Z = -2.10. Data is shown as median, quartile range and range. Significance of MWU-tests is given as *p < 0.05.

Behavior at the nest entrance and time spend inside the nest

In the *control* series, 15 % of all recruits travelling back to the nest unloaded their leaf disk in front of the nest entrance. The other 85 % entered the nest with their load. The same ratio was observed in the *treatment* series with 14 % dropping or transferring their fragment in front of the entrance and 86 % of all foragers entering the nest laden.

Camera recordings were analyzed to know how long laden foragers stayed inside the nest and if the duration might be dependent on the load they carried (Figure 6.8).

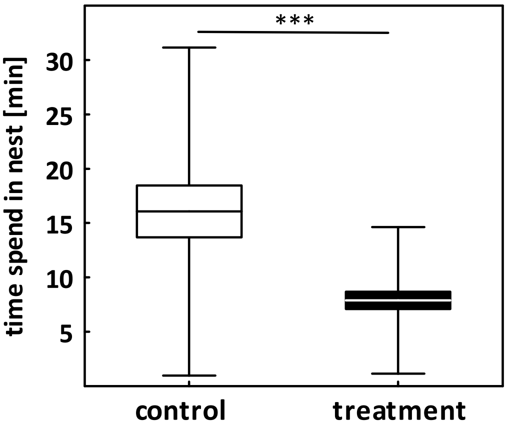

Figure 6.6: Time laden recruits stayed inside the nest [min] in *control* (☐), *treatment* (■). Recruits carried privet leaf disk known as suitable in *control* and as unsuitable in *treatment*. N (*con/treat*): 40/73; p < 0.001, t = -3.61, df = 111. Data is shown as mean ± SE and significance of unpaired t-tests is given as ***p < 0.001.

Recruits carrying a leaf disk known as unsuitable to the subcolony (treatment) stayed on average 7.9 ± 0.8 minutes inside the nest until they returned outside. In the control series, where recruits carried a leaf disk known as suitable to the subcolony, they stayed twice as long returning to the trail after 16.1 ± 2.4 minutes. Foragers that carried the reference plant species blackberry from the main food source (n = 46) spend 9.5 ± 1.3 minutes inside the nest. This duration is comparable to foragers carrying a privet leaf disk known as unsuitable (p = 0.11, t = 1.63, df = 117) and significantly shorter than the time spend inside the nest by foragers carrying privet in the control series (p = 0.03, t = -2.24, df = 84).

Discussion

In social insects, workers evaluate substrate suitability based on innate tendencies and personal experience, and individual preferences might additionally be modulated through social feedback mechanisms. In *Apis mellifera*, successful foragers return to the hive and the number of receiver bees interested in the load are an indirect measure of substrate quality (Seeley 1989). In leaf-cutting ants, as harvested plants do not directly serve as food for the workers but mainly as substrate for the symbiotic fungus, a third step, evaluation of the substrate in the fungus, is necessary to finally decide on substrate suitability. Some plant species initially evaluated by workers as suitable prove to be unsuitable for the fungus after their incorporation and are consequently rejected. Information about substrate suitability does not spread evenly throughout the colony. On the trail where the substrate was harvested, rejection already occurs after one day, but it takes 2-3 days for foragers to reject the substrate on adjacent trails (*Atta colombica*, Saverschek et al. 2010). Due to the time delay of the fungus' reaction to the unsuitable substrate, foraging at this particular food source might have been already fully established in the meantime. Transfer of information is therefore not only important during recruiting to a newly discovered food source, but also throughout normal foraging activity to and from an established food source.

Individuals had about 60 head-on encounters per individual on the way to the nest in both experimental series. There were no differences due to the perceived suitability of the substrate carried by the recruit probably because observed recruits returned from established food sources. Head-on encounter rates have been shown to serve as information source in the context of task allocation (Gordon & Mehdiabadi 1999; Pratt 2005; Greene & Gordon 2007), which is mainly of importance during recruitment. Traffic intensity on the trail affects head-on encounter rates of recruits on the way to the nest (Burd & Aranwela 2003). Howard et al. (1996) has shown in *Atta colombica* that workers seem to be influenced in their foraging decisions through the load carried by the majority of workers they encounter on their way out to the food source. Recruits were passively conditioned to prefer a substrate through the mere encounter of workers carrying said substrate.

Interestingly, mutual antennating could be only observed in about 50 % of all recruits on average twice per individual irrespective of the experimental series. This indicates that recruits were not actively looking for information by contacting nestmates. In a previous study I could already show a correlation between the percentage of unladen experienced foragers on the trail and the level of acceptance of the previously unsuitable substrate by naïve foragers after foraging together for eight hours (chapter 5), thus indicating an influence of unladen experienced foragers on plant preferences of naïve foragers. Besides the low occurrence of mutual antennation, all other observed active contacts were initiated by nestmates, either antennating the recruit or its load or biting into the leaf disk. Active seeking of encounters with nestmates has been previously described in outbound workers of *Atta cephalotes* (Burd & Aranwela 2003). In my study, nestmates approached recruits significantly more often if they had previous negative experience with the leaf disk carried by the recruit and overall, a significantly higher percentage of recruits received antennation and leaf bites under these conditions.

Regulation of foraging behavior based on substrate suitability is primarily known in the context of recruiting. Scouts laden with a leaf disk from a newly discovered food source meander slowly across the trail occasionally stopping and presenting their fragment to passing nestmates and often transferring their leaf fragment in the process (*Atta cephalotes,* Hubbell et al. 1980; *Atta vollenweideri*, Röschard & Roces 2003; *Acromyrmex lundi*, Geissler 2008; *Acromyrmex ambiguus*, personal observation). While presenting their load, scouts, like recruits, also receive antennation and bites from nestmates. Interestingly though, scouts carrying a leaf disk known as unsuitable to nestmates received significantly fewer bites than scouts carrying a leaf disk known as suitable. Due to the lack of received bites, scouts were less likely to return to the food site continuing their recruiting efforts (Geissler 2008). I can show that laden recruits on the way back to the nest receive more attention from their nestmates in the form of antennation and leaf bites carrying a substrate known to their nestmates as unsuitable than if they carry a suitable leaf fragment. One explanation for the contrary behavior of nestmates towards the carrier is the context in which the ants encounter each other. Scouts coming from a newly discovered food source seem to actively search for feedback from nestmates on the main trail. As scouts try to recruit nestmates to the newly discovered food source, raised attention from nestmates expressed as leaf bites can serve as positive reinforcement in this context. Recruits on the other hand travel back to the nest with few mutual antennations. Coming from a food source on a well established trail, the need of recruits to acquire information is low. Here, bites from nestmates probably disturb recruits in their task to return to the nest quickly and might lead to a lower acceptance of the substrate on the consecutive trip.

Surprisingly, interactions of nestmates cause no delay. The time it takes recruits to travel back to the nest with their load is the same (on average 3.6 min) irrespective of the perceived quality of their load even though the number of active contacts differs significantly. So interactions of nestmates seem to have no passive effect on the foraging efficiency of those recruits that carry the previously unsuitable substrate through delay of their return to the food source. Even though recruits encountering nestmates that had experienced privet as unsuitable (*treatment* series) received significantly more antennation and bites, recruits in the *control* subcolonies also received antennation and bites from nestmates. Therefore, a possible explanation could be that recruits are activated through antennation and bites to evaluate their load again, and once they reach the nest and enter it, they are more likely to look for feedback or information inside the nest. This would also explain the bites in the *control* series, as even though recruit and nestmates alike know the substrate (privet) as suitable, it is still less familiar than the main food source blackberry and might therefore raise more attention (Cherrett 1972).

Once recruits reach the nest, a great majority enters the nest irrespective of their load (~ 85 %) whereas the others leave their leaf disk in front of the nest entrance. Interestingly, foragers entering the nest with the supposedly unsuitable leaf disk stay around eight minutes, half as long as recruits with substrate known as suitable (16 minutes on average). Recruits in the *treatment* series, where subcolonies had received unsuitable privet the previous day, are probably more likely to immediately encounter the effects the substrate had caused on the fungus and leave the nest again with the newly obtained information. In the *control* subcolony, were substrate was suitable all along, recruits' were activated through interactions on the trail, maybe due to the fact that privet was still new to part of the foragers. Recruits were therefore motivated to look for changes in the fungus, which might explain the longer stay inside the nest. The fact that foragers also stay for an average of eight minutes if entering the nest with blackberry, supports this hypothesis as in this case, foragers already know about the suitability of their load as it has been their main food source.

It has already been shown that the presence of experienced foragers inside the nest influences substrate preferences in naïve foragers. Based on the results of this study it seems likely that recruits carrying a substrate known as unsuitable to nestmates are actively influenced in their substrate preferences through contacts on the trail. This seems likely as foragers sometimes travel long distances between nest and food source and are therefore away from the nest for long periods of time throughout the day. Communication between foragers on the trail leads to a faster distribution of information about substrate suitability among foragers therefore speeding up decrease in acceptance of substrate proven unsuitable by the symbiotic fungus.

GENERAL CONCLUSIONS

Deciphering the various parameters shaping the foraging patterns of leaf-cutting ant colonies has been a fascinating endeavor for a long time. Due to their association with their symbiotic fungus, foraging decisions of leaf-cutting ants are a multi-layered process. Host plant selection of individuals is not only based on the foragers' innate tendencies, but also influenced by their nestmates, previous experience and their symbiotic fungus. The feedback of the symbiotic fungus on the plant preferences of foragers has already been proven both in the laboratory (Knapp et al. 1990; Ridley et al. 1996; North et al. 1999) and in the field (Ridley et al. 1996) and mature colonies of *Atta colombica* still rejected the unsuitable substrate up to 14 weeks later (Saverschek et al. 2010).

My study attempted to define under which conditions foragers are influenced by their symbiotic fungus and which role individual experiences, as well as experienced nestmates play at different times after the initial rejection of a substrate. Here I present a schematic summary of the factors influencing individual foraging decisions about plant suitability (Figure 1). Individual foragers either accept or reject a newly encountered substrate based on innate tendencies. If the substrate is considered to be suitable, it is brought back to the nest and incorporated into the symbiotic fungus.

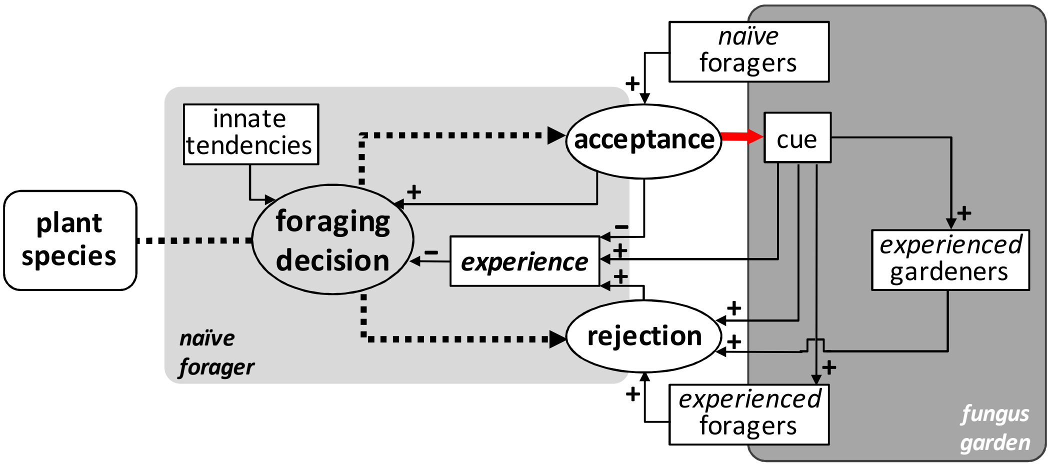

Figure 1: Schematic diagram of the factors influencing individual foraging decisions.

If it proves to be unsuitable, workers react to a cue in the fungus and reject the substrate within 24-48 hours. It has been postulated that a semiochemical from the fungus might be detected by the gardeners and passed on to the foragers (North et al. 1999). My results strongly suggest that foragers can learn either directly through evaluation of the fungus or indirectly through experienced gardeners. The nature of the fungal cue still remains elusive, but it has been shown that foragers decrease their acceptance only within two days after the initial incorporation of the substrate (Herz et al. 2008) leading to the conclusion that the cue is not present in the fungus after this time period anymore. After the reaction of the fungus provided the original information about the unsuitability of the substrate, experienced gardeners and foragers exist in the colony. Depending on the size of the colony and the number of leaf fragments of the unsuitable substrate accepted and incorporated, naïve foragers lacking experience with the substrate might initially still be present within the colony. If naïve foragers harvest the same substrate again, rejection occurs quicker as the feedback from the fungus is not necessary anymore. Experienced foragers already influence naïve foragers out on the trail leading to a decrease of acceptance within hours. Throughout time, as new workers emerge and join the workforce, the percentage of naïve foragers increases again. This variance in experiential backgrounds among workers concerning different plant species gives the colony the flexibility to try and test the substrate at a later point in time. Even it is suitable at a later point in time, possibly through seasonal changes in the chemical composition of leaves, naïve foragers still learn about its previous unsuitability from experienced gardeners inside the nest and reject the substrate within 24 hours. If the substrate incorporated into the fungus then proves to be suitable afterwards, acceptance slowly starts again.

Plant selection in leaf-cutting ants is regulated by several feedback loops that overlap or replace each other depending on the presence or absence of involved factors. The symbiotic fungus plays the central role in this two step evaluation of potential host plants. If the substrate is not already considered unsuitable due to innate tendencies of the ants, the feedback from the fungus decides on the further acceptance or rejection of the substrate. The fungus is therefore the basic source of information about plant suitability and the influence of experienced nestmates on naïve foragers under several conditions can be seen as additional factors. The existence of several pathways makes the system extremely robust and flexible at the same time. Information about the unsuitability of the substrate is present in the fungus for around 2 days. Through experienced workers, the information is still present in the colony after weeks, long after the effects on the fungus are not detectable anymore. The variation in the experiential background of foragers eventually allows the re-testing of substrate that previously proved to be unsuitable.

SUMMARY

Foraging behavior is a particularly fascinating topic within the studies of social insects. Decisions made by individuals have effects not only on the individual level, but on the colony level as well. Social information available through foraging in a group modulates individual preferences and shapes the foraging pattern of a colony. Identifying parameters influencing foraging behavior in leaf-cutting ants is especially intriguing because they do not harvest for themselves, but for their symbiotic fungus which in turn influences their plant preferences after the incorporation of the substrate. To learn about the substrates' unsuitability for the fungus, ants need to be able to identify the incorporated substrate and associate it with detrimental effects on the fungus. Odor is an important plant characteristic known to be used as recognition key outside the nest in the context of foraging. Chapter 1 shows that foragers are able to recall information about the unsuitability of a substrate through odor alone and consequently reject the substrate, which leads to the conclusion that inside the nest, odor might be enough to indentify incorporated substrate.

Identification of plant species is a key factor in the foraging success of leaf-cutting ants as they harvest a multitude of different plant species in a diverse environment and host plant availability and suitability changes throughout the year. Fixed plant preferences of individuals through innate tendencies are therefore only one factor influencing foraging decisions. On the individual as well as the colony level, foraging patterns are flexible and a result of an intricate interplay between the different members involved in the harvesting process: foragers, gardeners and the symbiotic fungus.

In chapter 2 I identified several conditions necessary for naïve foragers to learn about the unsuitability of substrate inside the nest. In order to exchange of information about the unsuitability of a substrate, the plant in question must be present in the fungus garden. Foragers can learn without own foraging experience and even without experiencing the effects of the substrate on the fungus, solely through the presence of experienced gardeners. The presence of experienced foragers alone on the other hand is not enough to lower the acceptance of substrate by naïve foragers in the presence of naïve gardeners, even if experienced foragers make up the majority of the workforce inside the nest. Experienced foragers are also able to reverse their previous negative experience and start accepting the substrate again.

The individual behavior of foragers and gardeners with different experiential backgrounds in the presence of suitable or unsuitable substrate inside the fungus chamber was investigated in chapter 3 to shed some light on possible mechanisms involved in the flow of information about substrate suitability from the fungus to the ants. Gardeners as well as foragers are involved in the leaf processing and treatment of the applied leaf patches on the fungus. If the plant material is unsuitable, significantly more ants treat the plant patches, but foragers are less active overall. Contacts between workers initiated by either gardeners or foragers occur significantly more frequent and last longer if the substrate is unsuitable. Even though experienced gardeners increase naïve foragers' contact rates and duration with other workers in the presence of suitable plant patches, naïve foragers show no differences in the handling of the plant patches. This suggests that foragers gain information about plant suitability not only indirectly through the gardening workers, but might also be able to directly evaluate the effects of the substrate on the fungus themselves.

Outside the nest, foragers influence each other the trail (chapter 4). Foraging in a group and the presence of social information is a decisive factor in the substrate choice of the individual and leads to a distinct and consentaneous colony response when encountering unfamiliar or unsuitable substrates. As leaf-cutting ants harvest different plant species simultaneously on several trails, foragers gain individual experiences concerning potential host plants. Preferences might vary among individuals of the same colony to the degree that foragers on the same trail perceive a certain substrate as either suitable or unsuitable. If the majority of foragers on the trail perceives one of the currently harvested substrates as unsuitable, naïve foragers lower their acceptance within 4 hours. In the absence of a cue in the fungus, naïve foragers harvesting by themselves still eventually (within 6 hours) reject the substrate as they encounter experienced gardeners during visits to the nest within foraging bouts. As foraging trails can be up to 100 m long and foragers spend a considerable amount of time away from the nest, learning indirectly from experienced foragers on the trail accelerates the distribution of information about substrate suitability. The level of rejection of a formerly unsuitable substrate after eight hours of foraging by naïve foragers correlates with the average percentage of unladen experienced foragers active on the trail. This suggests that unladen experienced foragers might actively contact laden naïve workers transmitting information about the unsuitability of the load they carry. Results from experiments were I observed individual laden foragers on their way back to the nest backed up this assumption as individuals were antennated and received bites into the leaf disk they carried. Individuals were contacted significantly more often by nestmates that perceived the carried leaf disk as unsuitable due to previous experience than by nestmates without this experience (chapter 6).

Leaf-cutting ants constantly evaluate, learn and re-evaluate the suitability of harvested substrate and adjust their foraging activity accordingly. The importance of the different sources of information within the colony and their effect on the foraging pattern of the colony depend on the presence or absence of each of them as e.g. experienced foragers have a bigger influence on the plant preferences of naïve foragers in the absence of a cue in the fungus garden.

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SUPPLEMENTS

Zusammenfassung

Besonders faszinierend ist das Furagierverhalten sozialer Insekten. Entscheidungen von Individuen haben nicht nur direkte Auswirkungen auf individueller Ebene, sondern auch auf Kolonieebene. Soziale Informationen modulieren individuelle Präferenzen beim Furagieren in der Gruppe und beeinflussen dadurch das Aktivitätsmuster der Kolonie. Die Identifizierung der Faktoren, die das Furagierverhalten beeinflussen, ist bei Blattschneiderameisen komplex, da sie nicht für sich, sondern für ihren symbiotischen Pilz furagieren. Dieser wiederum beeinflusst die Pflanzenwahl der Ameisen nach der Einarbeitung des Pflanzenmaterials in den Pilz. Um zu lernen, dass das eingebaute Substrat für den Pilz ungeeignet ist, müssen die Ameisen in der Lage sein, das bereits eingebaute Substrat zu identifizieren und mit den negativen Effekten auf den Pilz zu assoziieren. Duft ist ein bedeutendes Pflanzencharakteristikum, das außerhalb des Nestes als Identifizierungsmerkmal im Furagierkontext verwendet wird. In Kapitel 1 zeige ich, das Pflanzendüfte alleine ausreichen um Furageuren die Information aus dem Pilzgarten über die des Substrates ins Gedächtnis zu rufen. Furageure lehnen auf Grund des Duftes allein das Substrat bereits ab. Dies lässt den Rückschluss zu, dass Duft möglicherweise als Identifizierungsmerkmal des in den Pilz eingebauten Substrats ausreichend ist.

Die Identifizierung von Pflanzenarten ist ein wesentlicher Faktor des Furagiererfolgs bei Blattschneiderameisen, da diese eine Vielzahl unterschiedlicher Pflanzenarten ernten, deren Verfügbarkeit und Eignung sich im Jahresverlauf ändert. Angeborene individuelle Präferenzen sind daher nur einer von mehreren Faktoren, die die Furagierentscheidungen beeinflussen. Sowohl auf individueller als auch auf Kolonieebene sind die beobachteten Muster in der Furagieraktivität flexibel und das Ergebnis eines komplexen Wechselspiels aller Beteiligten im Furagierprozess: die Furageure, die Gärtnerinnen und der symbiotische Pilz.

In Kapitel 2 habe ich mehrere Bedingungen identifiziert, die notwendig sind, damit naive Furageure im Nest lernen können, das ein Substrat für den Pilz ungeeignet ist. Um Informationen über die Pflanzenqualität austauschen zu können, ist die Anwesenheit des Substrats im Nest erforderlich. Furageure können allein durch die Anwesenheit erfahrener Gärtnerinnen lernen, ohne eigene Furagiererfahrung und ohne die negativen Effekte des Substrats auf den Pilz

erfahren zu haben. Andererseits ist die Anwesenheit erfahrener Furageure allein nicht genug, um die Akzeptanz des Substrats durch naive Furageure zu verringern, wenn die Gärtnerinnen naiv sind, selbst wenn die erfahrenen Furageure die Mehrzeit der Tiere im Nest stellen. Erfahrene Furageure sind auch in der Lage, ihre früheren negativen Erfahrungen zu revidieren und das Substrat wieder zu akzeptieren.

Das Individualverhalten von Furageuren und Gärtnerinnen mit unterschiedlichem Erfahrungshintergrund in der Anwesenheit von geeignetem oder ungeeignetem Pflanzenmaterial im Pilz wurde in Kapitel 3 untersucht. Hierbei sollten mögliche Mechanismen des Informationsflusses vom Pilz zu den Ameisen aufgedeckt werden. Sowohl Gärtnerinnen als auch Furageure sind in die Bearbeitung des Blattmaterials involviert. Ist das Blattmaterial ungeeignet, wird es von signifikant mehr Ameisen bearbeitet, aber die allgemeine Aktivität der Furageure ist geringer als bei der Bearbeitung von geeignetem Substrat. Ist das Pflanzenmaterial ungeeignet, finden signifikant mehr und längere Kontakte zwischen den Ameisen statt. Die Anwesenheit erfahrener Gärtnerinnen hat keinen Einfluss auf die Bearbeitungszeit oder Frequenz des geeigneten Blattmaterials durch naive Furageure, sie haben aber einen Einfluss auf die von naiven Furageuren induzierten Kontakte. Diese sind in Anwesenheit von erfahrenen Gärtnerinnen häufiger und länger. Dies lässt vermuten, das Furageure sowohl direkt über den Zustand des Pilzes, als auch indirekt durch Kontakte mit erfahrenen Gärtnerinnen lernen, das ein Substrat für den Pilz ungeeignet ist.

Außerhalb des Nestes beeinflussen sich Furageure gegenseitig auf den Erntestraßen (Kapitel 4). Das Furagieren in der Gruppe und die dadurch zur Verfügung stehende soziale Informationen sind ein entscheidender Faktor in der Pflanzenwahl von Individuen und führt zu einer klaren und deutlichen Kolonieantwort bei unbekannten oder ungeeigneten Pflanzenarten. Da Blattschneiderameisen mehrere Pflanzenarten gleichzeitig auf unterschiedlichen Erntestraßen eintragen, unterscheiden sich Furageure in ihren individuellen Erfahrungen. Individuelle Präferenzen innerhalb einer Kolonie können sich so stark voneinander unterscheiden, dass eine Pflanze von unterschiedlichen Furageuren auf derselben Erntestraße sowohl als geeignet als auch als ungeeignet bewertet werden kann. Wenn die Mehrheit der auf der Erntestraße aktiven Furageure negative Erfahrungen mit dem Substrat hat und es als ungeeignet bewertet, dann verringert sich die Akzeptanz dieses Substrates durch naive Furageure ebenfalls signifikant innerhalb von 4 Stunden. Wenn die negativen Effekte im Pilzgarten nicht mehr zu detektieren sind lehnen naive Furageure in Abwesenheit von erfahren Furageuren das Substrat nach ungefähr 6 Stunden ab, da sie bei ihren Nestbesuchen auf erfahrene Gärtnerinnen stoßen. Da

Erntestraßen bis zu 100 m lang sein können und Furageure daher lange unterwegs sind, beschleunigt das indirekte Lernen durch erfahrene Furageure auf der Erntestraße die Verbreitung der Information über die Substratqualität innerhalb der Kolonie. Das Maß der Ablehnung des ursprünglich ungeeigneten Substrats durch naive Furageure nach 8 Stunden furagieren korreliert mit dem durchschnittlichen Prozentsatz an unbeladenen, erfahrenen Furageuren auf der Erntestraße. Dies lässt vermuten, dass unbeladene, erfahrene Furageure beladene naive Furageure aktiv kontaktieren und dadurch Informationen über das ungeeignete Substrat übermitteln. Ergebnisse von Individualbeobachtungen unterstützen diese Vermutung. In Kapitel 6 zeige ich, dass beladene Rekruten auf dem Weg zurück zum Nest signifikant häufiger von anderen Furageuren kontaktiert werden, wenn diese negative Erfahrungen mit der vom Rekruten getragenen Pflanzenart haben als wenn die Pflanzenart als geeignet bewertet wird.

Blattschneiderameisen bewerten, lernen und bewerten wieder die Qualität geernteten Substrats und passen ihr Furagierverhalten entsprechend an. Die verschiedenen Informationsquellen über die Pflanzenqualität innerhalb der Kolonie haben eine unterschiedliche Gewichtung abhängig von der An- oder Abwesenheit von einer von Ihnen. Zum Beispiel haben erfahrene Furageure in der Abwesenheit von negativen Effekten im Pilzgarten einen deutlich größeren Einfluss auf die Präferenzen naiver Furageure.

Publications

papers

Saverschek N, Herz H, Wagner M, Roces F 2010. Avoiding plants unsuitable for the symbiotic fungus: learning and long-term memory in leaf-cutting ants. *Animal Behaviour* 79: 689-698.

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Conference presentations

Mechanisms of Interspecific Interactions of Organisms, German Research Foundation (DFG), University of Würzburg, Germany » 2007 - talk

Collective and Individual Intelligence Symposium, Congress of the International Union for the Study of Social Insects (IUSSI), Washington D.C., USA » 2006 - talk

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Mechanisms of Interspecific Interactions of Organisms, German Research Foundation (DFG), University of Würzburg, Germany » 2005 – talk and poster

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Erklärung

Hiermit erkläre ich, die vorliegende Arbeit in allen Teilen selbstständig angefertigt und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet zu haben.

Die Dissertation wurde bisher weder vollständig noch teilweise einer anderen Hochschule mit dem Ziel einen akademischen Grad zu erwerben, vorgelegt.

Von der Universität Göttingen wurde mir 2004 der akademische Grad Diplom Biologin verliehen. Weitere akademische Grade habe ich weder erworben noch versucht zu erwerben.

Würzburg, den