CHAPTER 9

General Discussion

Orientation within a complex web of infochemicals

This chapter provides an overview of what has been learnt about the infochemicals involved in the multitrophic interactions of the pteromalid parasitoid *L. distinguendus*, the hosts and host-associated organisms as well as the host's food plant by combining main results and conclusions of this thesis with those of recently published studies. Research reported here embraces both the mate finding and mating behaviour of males and the host finding behaviour of females, with special emphasis on the chemical nature and functional role of compounds affecting the intra- and interspecific interactions in the storage environment. Findings on *L. distinguendus* are summarised and discussed in a broader ecological context and compared with other parasitoid species, in particular with *N. vitripennis*. The outcome of this is a presentation of a complex and fascinating web of infochemicals to which the parasitoid is exposed in its "natural" habitat (Fig. 1). Although the chemical communication of *L. distinguendus* has been extensively examined in recent years as hardly ever done in other parasitoid species, the current knowledge still reflects only a part of the reality, since infochemical webs are expected to be more complex than food webs (Vet, 1999). Nevertheless, it is very likely that mechanisms mainly investigated in the laboratory are also expressed in nature because the monotonous and man-made situation in the storage environment where parasitoids of *L. distinguendus* commonly occur seems to be very close to the laboratory situation (Steidle and Reinhard, 2003).

Short-range sex pheromone stimulates mating behaviour

In *L. distinguendus* the stereotypical elements of the male courtship behaviour have been shown to be elicited by a female-derived sex pheromone (Ruther et al., 2000). The range of pheromonal activity is restricted to the direct vicinity of the females. However, apart from some basic aspects nothing was known about pheromonal

communication in this pteromalid wasp so far. Studies presented in this thesis showed that the courtship pheromone consists of a series of cuticular hydrocarbons (chapter 2 and 3) which generally have a low volatility and thus may explain why filter papers treated with pheromone extract of *L. distinguendus* females remained active over several weeks (Ruther, personal communication). Comparison of hydrocarbon profiles from active and inactive life stages using multivariate statistical methods strongly indicates that particularly methyl-branched hydrocarbons, such as 3-MeC27, 3-MeC29, 3,7-DiMeC27 and 3,7 DiMeC29, but also some monoenes, such as C27:1(9) and C29:1(7), are involved in the stimulation of sexual responses in males (chapter 3). Ultimate proof about the role of individual compounds in the pheromonal message can only be provided by performing experiments with synthetic compounds or with hydrocarbons isolated from bioactive extracts. However, despite recent advances in analytical techniques this requires an enormous effort because most hydrocarbons are not commercially available and it still remains difficult to synthesise the individual hydrocarbons or to collect them by chromatography (Howard, 1993). In addition, many sex pheromones of insects have been shown to consist of a number of components that act in synergy when presented together in a particular ratio (Wyatt, 2003). With some exceptions mainly for dipteran pheromones (e.g., Carlson et al., 1971; Nemoto et al., 1994; Doi et al., 1997), the knowledge of the role of individual hydrocarbons in the chemical message of other insects is still limited. As part of this thesis, several hydrocarbon components assumed to elicit the sexual response in *L. distinguendus* males were isolated from bioactive hydrocarbon fractions by preparative GC-MS and tested for pheromonal activity. Males did not respond when the hydrocarbons were offered alone, but were arrested when the GC-fraction was presented in combination with an inactive hydrocarbon profile of a male (data unpublished). Results suggest that the response of *L. distinguendus* males is context-dependent and requires not only the bioactive compounds but also a species-specific cuticular hydrocarbon profile as background odour. However, more complex elements of the characteristic courtship behaviour (e.g., wing fanning) were not elicited by the GC-fraction. Chemical analysis of the tested fraction showed that not all hydrocarbons considered to be necessary for behavioural activity were present or only in low concentrations.

The role of hydrocarbons as courtship pheromones of parasitic wasps

Hydrocarbons including *n*-alkanes, alkenes and methyl-branched components are the major compounds of insect epicuticular lipids (Nelson and Blomquist, 1995). Numerous biochemical, physiological, ecological and behavioural aspects of insect cuticular hydrocarbons have been investigated so far. It is becoming increasingly clear that they evolved not only as protective water barrier and defence against microorganisms (Buckner, 1993) but are also of vital importance in the chemical communication of many insects, in particular of social ones (Howard and Blomquist, 1982, 2005). Cuticular hydrocarbons have been shown to function as species, caste, kin and host recognition cues (e.g., in Hymenoptera, Isoptera) or facilitate sexual behaviour as sex attractants, aphrodisiacs or anti-aphrodisiacs (e.g., in Coleoptera, Diptera, Lepidoptera) (Blomquist et al., 1993). Findings presented in this thesis clearly demonstrate that hydrocarbons are also involved in the sexual communication of parasitic Hymenoptera (chapter 2, 3, 5). In both pteromalid species investigated hydrocarbons serve as short-range or contact pheromones to stimulate the courtship behaviour of males (Steiner et al., 2005; Steiner et al., 2006). In *N. vitripennis* mainly quantitative differences in the cuticular hydrocarbon profiles of males and females seem to be the chemical basis of the sex pheromone. In contrast, pheromone activity in *L. distinguendus* was particularly characterised by a series of compounds exclusively found in the cuticular extracts of active life stages. Since a few other studies have provided evidence for the function of cuticular hydrocarbons as courtship pheromones (Syvertsen et al., 1995; Sullivan, 2002), their use in mating behaviour might be a common feature at least in the Pteromalidae, but possibly also in many other taxa.

Release of the courtship pheromone during pupal development

The spatial distribution of hosts strongly influences the mating system of many parasitic Hymenoptera (Godfray and Cook, 1997). *L. distinguendus* is known to parasitise larvae of several beetle species that lay their eggs in clumps. Thus, male and female parasitoids develop in the near vicinity of one another and commonly mate at the site of emergence. The study described in chapter 2 demonstrates that the short-range sex pheromone of *L. distinguendus* does not only elicit the courtship behaviour but also enables the accurate detection of conspecifics about to emerge. Experiments revealed that the bioactive compounds are already

produced during pupal development of females. Interestingly, searching males are able to recognise the pheromone through the thin and often porous shell of grains containing a female. This mechanism seems to be advantageous for both sexes. Since *L. distinguendus* females mate only once under normal circumstances (chapter 4), selective pressure may favour males that are able to find receptive females earlier than competitors. Waiting for females to emerge from the grain might be therefore more promising than searching for already emerged females because this strategy increases the probability of encounters with virgins. Females, on the other hand, might benefit from releasing the sex pheromone already during development since it enables mating before having left the emergence site to search for new host patches. A comparative study on the pheromone chemistry of *N. vitripennis* revealed that the release of sex pheromones by immature stages is not a general phenomenon in parasitoids (chapter 5). In this pteromalid species, the pheromone activity was found only in adult females.

Differences in the beginning of pheromone production might be explained by the different life histories of both species. *N. vitripennis* develops in groups of 10 to 20 individuals, by majority females, inside the host fly puparium (van den Assem et al., 1980). Males emerge shortly before females and commonly remain on the surface of the puparium to copulate with their emerging sisters (van den Assem, 1986). In contrast, in *L. distinguendus* only a single parasitoid emerges from the parasitised grain. Thus, males have to leave the site of emergence and search for females within a larger area of the host patch. Mechanisms reliably indicating the presence of females about to emerge might thus be more important in solitary than in gregarious species. So far, the aspect of pheromone production in immature stages has been widely neglected in the literature. A study on the solitary pteromalid species *Anisopteromalus calandrae* demonstrating the pre-emergence release of a courtship pheromone seems to support this hypothesis (Yoshida, 1978). However, there is also an example demonstrating pheromonal activity in pupal stages of the gregarious braconid species *Apanteles glomeratus* (Tagawa, 1977).

Female sex pheromone in immature males

Somewhat surprisingly, compounds inducing sexual response in adult males of *L. distinguendus* are not only released by immature females but also males (Steiner et al., 2005) (chapter 2). Soon after emergence, however, pheromone activity in

males was shown to disappear. Further bioassays demonstrated that males, when searching for mates, are not able to recognise the parasitoid sex inside the grain and are equally arrested on grains containing either females or males. It was thus suggested that developing males release the female courtship pheromone to fool their already emerged competitors. By distracting rivals away from actual females, males inside the grain may increase the probability of mating with these females after emergence and may therefore compensate the disadvantage of late emergence to a certain degree. Thus, this phenomenon was suggested to be a novel case of pre-emergence chemical mimicry.

An important precondition for the evolution of mimetic resemblances is that deception leads to a higher fitness of the mimic. However, this proof might be hardly practicable in most cases (Dettner and Liepert, 1994). To confirm the hypothesis of the pre-emergence chemical mimicry in *L. distinguendus*, the fitness criterion was reduced to a more testable feature. It was tested whether later emerging males at least increase their probability of mating by chemically mimicking the female-derived sex pheromone. For this purpose microcosm experiments were conducted (data unpublished). Single males and a group of 20 females[4] were placed in Petri dishes that were filled with a mixture of differently treated grains. The two test treatments contained healthy grains, host-infested grains and either 16% or 24% parasitised grains with *L. distinguendus* males about to emerge. In contrast, control treatments did not contain parasitised grains but only healthy and host-infested grains. In these microcosm experiments, males had the chance to find the females within the differently treated grains of test and control Petri dishes and mate with them for 10 hours. Based on the hypothesis pre-emergence chemical mimicry, it was expected that males of the both test treatments would mate with a lower number of females when compared to the control. However, the number of mated females in the test treatments did not differ significantly from the control, although at least in the Petri dishes with 16% parasitised grains a tendency was detectable (χ^2 = 2.99, P = 0.084). Surprisingly, in the Petri dishes with a higher degree of parasitisation where the effect of deception was assumed to be at highest, the number of mated females was almost similar to the control (χ^2 = 0.28, P = 0.598). A possible explanation is that the design of the microcosm experiments was not suitable to demonstrate potent advantages of pre-

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[4] A number of 20 females has been shown to be more than a single male can fertilise within the duration of the experiment (10 h) (chapter 4).

emergence males by the release of the sex pheromone. Although all Petri dishes were darkened with aluminium foil, parasitoids were sometimes observed to meet each other at the top of the Petri dishes where males were able to mate successively with the females. Thus, further experiments are imperative to finally clarify whether pre-emergence males effectively gain fitness by releasing the female odour.

An alternative explanation why immature stages of male and female insects elicit sexual response in adults was given by Haynes et al. (1992). The authors demonstrated that males of *Cyclocephala lurida* (Coleoptera: Scarabaeidae) were attracted by conspecific larvae of either sex. The behavioural response to the larvae was excluded to be functional as shown in other insects (e.g., Gilbert, 1976), since immature stages of *C. lurida* live above ground feeding on grass roots and do not co-occur with adults. It was thus proposed that the pheromone communication in this scarab beetle is derived from larval chemicals that are lost in adult males but retained by the females. This might also be true for *L. distinguendus*. It is conceivable that the courtship pheromone has evolved from a series of hydrocarbons having no communicative but a physiological function in the pupal stages of either sex, such as the prevention of desiccation (Lockey, 1988). Originally, male and female parasitoids might have possessed the same cuticular hydrocarbon profiles allowing for species rather than sex recognition. However, males adapted to using hydrocarbons for detection of conspecifics will increase the probability of finding receptive females because of the strongly female-biased sex ratio. Nevertheless, regular homosexual courtship interactions would be a consequence of males and females sharing the same recognition cues. Since male-male courtship interactions prevent the involved individuals from courting for females, selection might have favoured the evolution of a mechanism in freshly emerged males that renders their hydrocarbon profiles unattractive for male conspecifics. Such a mechanism has been demonstrated in this thesis (chapter 2) because it was shown that males of *L. distinguendus* loose pheromone activity within 32 h after emergence due to an active process (e.g., by decomposition or relocation of involved compounds) rather than passive evaporation. This indicates that there might be a selective pressure on the adult males to get rid of the courtship mediating compounds. Future studies should examine whether freshly emerged males of *L. distinguendus* that still release the female-derived sex

pheromone really gain mating disadvantages through the molestation by other males when competing for receptive females.

Use of infochemicals in mate location

Godfray (1994) proposed that the evolution of costly long-range pheromones for mate attraction might be less common in haplodiploid parasitoids where even females that fail to mate gain fitness by producing male offspring from unfertilised eggs. However, most sex pheromones that have been demonstrated in parasitic Hymenoptera are volatile (for details see Kainoh, 1999). In almost all cases, female parasitoids attract males. However, studies of this thesis provide evidence that the males but not the females of *N. vitripennis* release mate attractants, identified as a mixture of the diastereomers (4*R*,5*R*)- and (4*R*,5*S*)-5-hydroxy-4-decanolide (HDL) (chapter 6). Interestingly, the response of the females to HDL was controlled by their mating status. Receptive females were strongly attracted to the male-produced pheromone, whereas mated ones avoided HDL and responded neutrally with increasing time after mating.

Males of *L. distinguendus*, a species where neither males nor females seem to attract the opposite sex, have found another valuable way of orientating towards females from a distance. They respond to volatiles emitted by the larval faeces of the host *S. granarius* that are used as well by females for host location (chapter 7). In addition, both sexes are attracted to chemicals released by host-associated mites when associatively learnt during mating and host encounters, respectively (Ruther and Steidle, 2000). Since *L. distinguendus* males unlike females do not feed on the hosts, it is likely that males use these host-associated odours exclusively for mate finding and thus, they meet the definition of sexual kairomones (Ruther et al., 2002). In this context, host-associated volatiles seem to have two main functions. Firstly, males searching for mates in the surrounding of their emergence site may use these chemicals to get information about their position within the host habitat. The perception of decreasing amounts of host odours reliably signals the males that they are about to leave the area of host infestation and thus the area of female emergence. Secondly, it is also possible that males of *L. distinguendus* disperse and attempt to find additional mating opportunities outside their own emergence site. By using the same chemical cues as females do for host location, males presumably increase the probability of encounters with

receptive females at possible oviposition sites (Ruther and Steidle, 2000). The mechanisms described might be also true for *N. vitripennis* since olfactometer experiments demonstrated that both sexes were strongly attracted to volatiles derived from the puparia of their host *Lucilia caesar* (unpublished data). The strategy to search for sites where males can expect to find females (emergence, oviposition and feeding sites) is well-known from parasitoid species where the sexes develop in different parts of the environment (Godfray and Cook, 1997). Though little is known about the cues that guide the males to these sites, again volatiles emitted by the hosts (Matthews et al., 1979) or host-associated organisms (Madden, 1968; Spradbery, 1970) seem to play a crucial role in mate finding.

Volatile chemicals used for (host) habitat and host location

Numerous studies have investigated the behaviour and infochemicals involved in the host selection process of *L. distinguendus*. The parasitoid is known to attack immature stages of the granary weevil *S. granarius* and several other non-related pests that develop in stored plant products (Steidle and Schöller, 1997). As in many other parasitoid species, females of *L. distinguendus* do not search randomly for hosts but use in particular chemical cues for orientation. Volatiles emitted by stored grains and seeds have a slightly arresting effect on foraging females even in the absence of the host suggesting that these components, in addition to physical parameters such as humidity (Steidle and Reinhard, 2003), are used by the parasitoid for potential host habitat location (Steidle et al., 2001).

After having reached the storage environment, females have to search over at least some distance for the hosts. The ability to locate areas of host infestation seems to be highly developed in *L. distinguendus* since the parasitoid has been reported to find and parasitise host-infested grains up to 4 m deep in stored grain (Steidle and Schöller, 2000, 2002). Odours derived from the larval faeces of *S. granarius* and other hosts of *L. distinguendus* (*Rhyzopertha dominica*, *Callosobruchus maculatus*) direct the females to the hosts (Steidle and Schöller, 1997; Steidle et al., 2001; Steidle et al., 2003) (chapter 7). Faeces are often pushed out by the weevil larvae through small holes in the infested grains and thus cover the outer surface (Steidle and Fischer, 2000). Interestingly, an initial chemical analysis of faecal headspace extracts from *S. granarius* revealed that the major compounds, particularly neral, geranial, neryl formate and tridecane, did not originate from the host itself but from

astigmatid mites that often co-occur with hosts of *L. distinguendus* (Ruther and Steidle, 2000). Some of the chemicals have been described as compounds of the alarm pheromone in the mould mite *Tyrophagus putrescentiae* (Kuwahara et al., 1975, 1979; My-Yen et al., 1980). These mites are particularly abundant at sites of intense primary infestation by pest beetles (Sinha, 1961).

Female parasitoids responded to synthetic mite volatiles when previously conditioned by associative learning during encounters with hosts (Ruther and Steidle, 2000). In contrast, mite chemicals had no effect on naïve females (Steidle et al., 2003). The ability of parasitoids to learn profitable cues of the surrounding environment to improve their host finding and parasitism rate was shown in several studies (reviewed by Vet et al., 1995). Thereby, the ranking of chemical stimuli to which parasitoids originally show at most limited response can remarkably increase during the foraging process when associated with the presence of the host (Godfray, 1994). Results of recent work suggest that *L. distinguendus* could be an ideal candidate for the investigation of learning ability, memory duration and memory structure in parasitic Hymenoptera as well as the implications on the foraging behaviour (Collatz et al., 2006; Müller et al., 2006).

Further studies demonstrated that larval faeces also emit volatiles that are attractive to naïve females (Steidle and Schöller, 1997; Steidle et al., 2001). Thus, the behavioural response of the females to these chemicals has to be innate. However, it was not clear whether the attractive volatiles originate from the host-associated mites or from the faeces. By using rearing conditions for the weevil cultures preventing secondary infestation by moisture-sensitive mites, it could be shown that odours of the larval faeces themselves attract naïve females and thus, are crucial for the effective location of hosts within the storage environment (chapter 7). Faeces are well-known to act as highly reliable indicators for the presence of hosts and are extensively used by parasitoids for host location and host recognition (e.g., Takabayashi and Takahashi, 1989; Mattiacci and Dicke, 1995; Agelopoulos et al., 1995; Alborn et al., 1995).

Bioassays using fractionated headspace extracts from larval faeces of *S. granarius* revealed that the host-associated kairomone is composed of a complex blend of compounds with different polarities. It is assumed that generalist parasitoids such as *L. distinguendus* innately use general rather than specific cues for foraging

processes and thus, the host-range of parasitoids seems to be constrained by chemical similarities of the hosts (Vet and Dicke, 1992; Godfray, 1994). Hence, it is likely that attractive volatiles in the larval faeces of *S. granarius* might be also present in the faeces of all other hosts of the parasitoid. These expected similarities might be helpful for identification of the still unknown nonpolar components of the foraging kairomone used by *L. distinguendus* females.

Apart from general host finding cues of mites and larval faeces, however, females of *L. distinguendus* may also use specific chemical cues since they innately respond to dominicalure 1 and 2 (Steidle et al., 2003), a male-released aggregation pheromone of one of their hosts, the lesser grain borer *Rhyzopertha dominica* (Williams et al., 1981). On the other hand, experiments with sitophilate, the aggregation pheromone of *S. granarius* (Phillips et al., 1989), did not attract *L. distinguendus*. According to the authors, the most likely explanation for the use of such specific cues might be that *L. distinguendus* was originally specialised on *Rhyzopertha dominica* and became a generalist only later by entering the storage environment and expanding the host-range. However, nothing is known about the original host so far (Kaschef, 1964).

Mould volatiles used for host habitat assessment

The ability of female parasitoids to discriminate between suitable and suboptimal host habitats is of great importance since optimal developing conditions for the offspring translates into parental fitness. Intense growth of moulds in stored grain can lead to extinct areas of extreme environmental conditions (Sinha, 1961; Sinha and Wallace, 1966). Studies of this thesis demonstrated that the fitness of *L. distinguendus* was drastically lower on hosts from moulded weevil cultures than in non-moulded ones (chapter 8). However, *L. distinguendus* females are able to detect and avoid host habitats negatively affected by the growth of mould by using fungal volatiles. A major component of the fungal odour bouquet, identified as 1-octen-3-ol, strongly repelled *L. distinguendus* females in the olfactometer. However, larval faeces from moulded weevil cultures containing considerable amounts of 1-octen-3-ol did not repel female parasitoids but were clearly avoided when offered simultaneously with faeces from non-moulded weevil cultures. These results suggest that foraging *L. distinguendus* females concentrate their host search
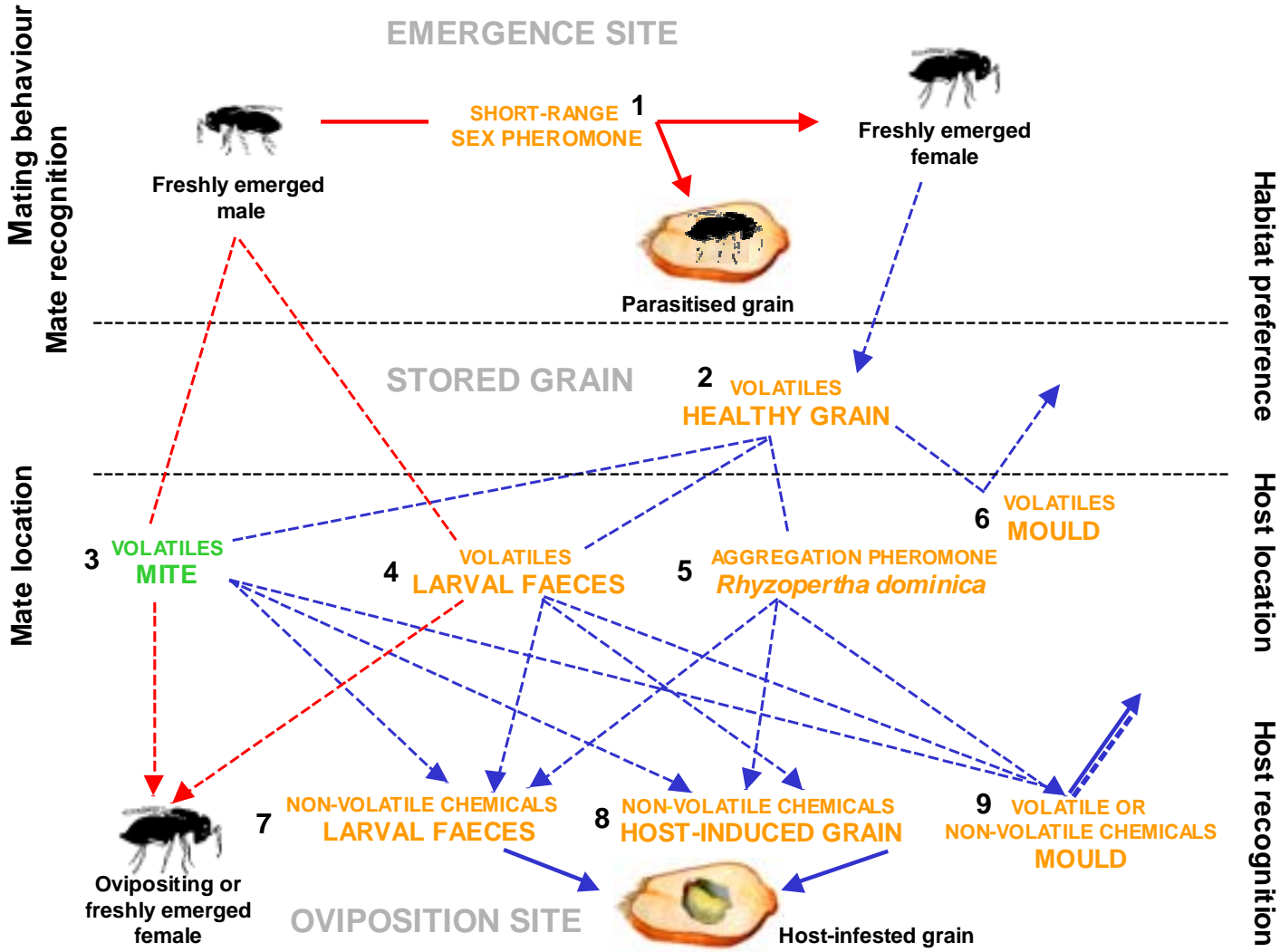
activity on non-moulded patches promising the highest reproductive success but will also orientate towards suboptimal patches in absence of non-moulded areas.

Non-volatile chemicals used for host recognition and host stage selection

Having entered a suitable host habitat, *L. distinguendus* must be able to distinguish between infested and uninfested grains for parasitisation. Again, compounds originating from the larval faeces are crucial for host recognition (Steidle and Ruther, 2000). However, in contrast to the long-range orientation that is mediated by faecal volatiles, host recognition behaviour is stimulated by non-volatile compounds (Steidle, 2000). Physical stimuli, such as a three-dimensional structure, play a minor role in *L. distinguendus* but only in combination with chemicals from the faeces. When encountering infested grains, female parasitoids intensely explore the grain by performing a series of behavioural elements including antennal drumming, abdominal tapping and ovipositor drilling that precede oviposition (Steidle, 2000). Very similar behavioural sequences are also observed in several other parasitoid species (e.g., Suzuki et al., 1984; Meiners et al., 1997). Chemical analyses and bioassays of fractionated faecal extracts revealed that mixtures of α-tocopherol, β-tocopherol, β-tocotrienol, cholesterol, ergostenol and β-sitosterol stimulated host recognition behaviour in female parasitoids (Steidle and Ruther, 2000).

A recent study by Steidle et al. (2005) provided evidence, that *L. distinguendus* females may also use chemicals for host recognition that are released by the grain after infestation by *S. granarius.* It is well established that plants modify their volatile blend as a result of herbivore feeding or oviposition activity and in this way "call for help" since these volatiles attract natural enemies of the herbivores (Rutledge, 1996; Steidle and van Loon, 2002; Hilker and Meiners, 2006). Remarkably, already reproductive plant stages, i.e., grains, seem to produce such herbivore-induced synomones as suggested by the mentioned study. Further results indicate that this plant response actually translates into increased fitness. The authors demonstrated that infested grains can increase their viability significantly when herbivore larvae were killed, for example by parasitoids, within the first three larval stages. In contrast, grains in which larvae developed up to the fourth stage or even later did not develop into a mature plant. This leads to the assumption that not only the

EMERGENCE SITE

1 SHORT-RANGE SEX PHEROMONE

Freshly emerged male

Freshly emerged female

Parasitised grain

MATE FINDING PROCESS

Mating behaviour

Mate recognition

Mate location

STORED GRAIN

2 VOLATILES HEALTHY GRAIN

3 VOLATILES MITE

4 VOLATILES LARVAL FAECES

5 AGGREGATION PHEROMONE *Rhyzopertha dominica*

6 VOLATILES MOULD

7 NON-VOLATILE CHEMICALS LARVAL FAECES

8 NON-VOLATILE CHEMICALS HOST-INDUCED GRAIN

9 VOLATILE OR NON-VOLATILE CHEMICALS MOULD

Ovipositing or freshly emerged female

OVIPOSITION SITE

Host-infested grain

HOST SELECTION PROCESS

Habitat preference

Host location

Host recognition

Fig. 1 (left side) Orientation of a parasitoid within a complex web of infochemicals. Movements of male and female *L. distinguendus* during mate (red arrow) and host (blue arrow) finding between the emergence site of the parasitoids, areas of uninfested stored grain and the oviposition site of the females depending on different sources of volatile (dashed line) and non-volatile (continuous line) chemicals. Infochemicals that have to be learnt by *L. distinguendus* are presented in green colour, whereas compounds used innately by the parasitoid are presented in orange colour. **1** Mating occurs commonly at the site of emergence. The female-derived sex pheromone (a series of cuticular hydrocarbons) causes arrestment and elicits stereotypical courtship behaviour in males (chapter 2 and 3) (Ruther et al., 2000; Steiner et al., 2005). In addition, compounds enable males to recognise grains containing conspecifics about to emerge (chapter 2) (Steiner et al., 2005) **2** After mating, female parasitoids leave the site of emergence. Volatiles originating from healthy grains are used to locate potential host habitats (Steidle et al., 2001a) **3** Males may also disperse and are able to use the same chemical cues for mate finding as females use for host finding. Volatiles emitted by astigmatid mites (neral, geranial, neryl formate, tridecane) co-occurring with the hosts can be associatively learnt by both sexes to locate new oviposition and mating sites, respectively (Ruther and Steidle, 2000; Steidle et al., 2003) **4** Volatiles (a blend of polar and nonpolar compounds) emitted by the larval faeces of *S. granarius* (and other hosts) mediate long-range orientation of male and female parasitoids during mate and host finding, respectively (chapter 7) (Steidle and Schöller, 1997; Steidle et al., 2001a) **5** Apart from general host finding cues, *L. distinguendus* is also able to respond to species-specific compounds. To locate host habitats of *Rhyzopertha dominica* female parasitoids make use of the beetle's aggregation pheromone (Steidle et al., 2003) **6** Chemical cues can not only attract but also deter parasitoids from entering oviposition sites. 1-Octen-3-ol originating from fungi is used by the females for host habitat assessment to avoid negative fitness consequences due to secondary mould infestation (chapter 8) **7** After having reached the host habitat, parasitoids must be able to distinguish between infested and uninfested grains for parasitisation. Non-volatile chemicals originating from the larval faeces of *S. granarius* (α-tocopherol, β-tocopherol, β-tocotrienol, cholesterol, ergostenol, β-sitosterol) stimulate the stereotypical host recognition behaviour in females (Steidle, 2000; Steidle and Ruther, 2000) **8** Alternatively, non-volatile chemicals released by host-infested grains can be used for host recognition, especially in areas of low humidity (Steidle et al., 2005) **9** Chemicals associated with mould infestation of infested grains cause an alleviated host recognition behaviour putatively resulting in a reduced number of eggs (chapter 8).

attracted parasitoids but also the grains might benefit from the release of the compounds. It is expected that females of *L. distinguendus* make increasingly use of these herbivore-induced synomones in situations where host larval faeces do not cover the surface of the infested grains. This might be especially the case when hosts develop in areas of low humidity and do not push out the faeces from the inside of the grain (Steidle et al., 2005).

Females of *L. distinguendus* generally prefer to parasitise older host stages (third and fourth instars) (Yoo and Ryoo, 1989; Steidle and Fischer, 2000), most likely

because they provide optimal nutrient supply for the parasitoid offspring. In addition, the evaluation of the host size seems to play also a key role in the sex allocation. Since *L. distinguendus* gains a higher fitness from large daughters rather than from large sons (van den Assem et al., 1989), females preferably lay fertilised eggs on large hosts to produce females. Mechanisms mediating the host stage selection in *L. distinguendus* were recently investigated (Steidle and Fischer, 2000). It was found that the degree of drumming and drilling behaviour was significantly affected by the amount of larval faeces accumulating in the weevil-infested grains. Since older host larvae produce larger amounts of faeces, parasitoids seem to be able to recognise them by assessing the quantity of the larval faeces.

Conclusions and future perspectives

The study of infochemicals playing a decisive role in the life of parasitic Hymenoptera contributes to our understanding of how these ecologically and economically important insects live, how they influence the population dynamics of herbivores and plants in multitrophic interactions. This research may help to develop environmentally safe methods of pest control employing parasitoids as effective natural enemies. Since hardly anything was known about of the chemicals involved in the mate finding and mating behaviour of the Pteromalidae, the investigations presented in this thesis give new and interesting insights into the sexual communication of this biologically diverse taxon.

It was shown that cuticular hydrocarbons serve as short-range (*L. distinguendus*) and contact (*N. vitripennis*) pheromones released by the females to elicit the male courtship behaviour. Since this was also reported for another pteromalid species (Sullivan, 2002), further efforts should be undertaken to investigate whether the use of cuticular hydrocarbons as courtship pheromones might be a general feature in the Pteromalidae. Thereby, the combined use of behavioural, chemical and statistical methods as practised in this work might be exemplary for future studies on parasitoid pheromone chemistry.

Furthermore, this work demonstrated that the bioactive compounds do not only mediate courtship behaviour in *L. distinguendus* but also allow searching males to

reliably detect grains containing females about to emerge. This is possible because female parasitoids produce the pheromone already during pupal development. Thus, it would be interesting to study whether the release of courtship pheromones in immature stages can be also found in other parasitoid species. And if so, it has to be investigated which similarities in the life histories of the species could have favoured the evolution of this phenomenon. In this context, the hypothesis should be proved whether the release of the courtship mediating compounds during pupal development is more advantageous for solitary than for gregarious species and thus should be mainly found in the parasitoid of the former life history.

L. distinguendus cultures kept under relatively constant laboratory conditions seem to show a hydrocarbon profile that is very similar between the generations (personal observations) and might be thus genetically determined. However, several non-genetic factors, such as the supply of nutrients, might have also an effect on the hydrocarbon production in insects. To date, this has been rarely investigated in parasitic Hymenoptera. The few studies addressing this question so far, found mainly limited correlation between the cuticular hydrocarbon profile of the hosts and that of the parasitoids (Howard and Liang, 1993; Howard, 2001). However, it was demonstrated for several parasitoid species that their fatty acid profiles were almost identical to those of their host bark beetles from which they were reared (Kudon and Berisford, 1981). Since fatty acids serve as precursors in the biosynthetic pathway of hydrocarbons (Blomquist et al., 1987), a change of the host species and thus of the host fatty acid composition might also influence the hydrocarbon profile of the parasitoids. *L. distinguendus* is known to parasitise several beetle species from 5 different families (Steidle and Schöller, 1997). Thus, future studies should address the question whether the cuticular hydrocarbon profile of *L. distinguendus* is affected by its hosts. Since the short-range sex pheromone consists of a series of hydrocarbons, it would be furthermore interesting to know whether a variation of the bioactive compounds also influences the female attractiveness to males during courtship.

In contrast to the increasing knowledge of the role of female-derived sex pheromones in mate attraction and mating behaviour of parasitic Hymenoptera, little is known about pheromones released by males. In this thesis, the determination of the chemical nature and functional role of a male-produced attractant in *N. vitripennis* was presented. Since the males release the chemicals in

larger quantities, it might be possible to elucidate the definitive location of the sex pheromone gland in the abdomen of the wasps. (+)-Vernolic acid has been reported to be the precursor of HDL-stereoisomers in microorganisms (Garbe and Tressl, 2003). Thus, it would be interesting to know whether HDL is biosynthesised by the same pathway in insects. The use of labelled precursors might be an appropriate approach to answer this question. Future studies addressing the regulation of pheromone production and perception in parasitic wasps should embrace novel techniques of molecular biology and genetics (Tittiger, 2004). The genome of *N. vitripennis* is about to be completely sequenced by the Nasonia Genome Project (for details visit http://www.hgsc.bcm.tmc.edu/projects/nasonia/) and this will probably provide new tools in the study of the genetic mechanisms involved in the chemical communication of parasitic wasps.

Results of numerous studies investigating important aspects for the evaluation of natural enemies in biocontrol suggest that *L. distinguendus* could be a promising candidate for the protection of stored foodstuff from insect infestation (e.g., van den Assem et al., 1989; Shin et al., 1994; Ryoo et al., 1996; Steidle and Schöller, 1997; Steidle, 1998; Ruther and Steidle, 2000; Steidle and Fischer, 2000). The knowledge of the chemical nature of host-related kairomones mediating the mate and host finding of *L. distinguendus* could be applied for monitoring the parasitoid population density in the pest-infested storage environment, retaining the parasitoids in the target area and providing maximum rates of parasitisation even at lower host densities (Powell, 1986; Godfray, 1994; Cox, 2004). Thus, following up the initial characterisation of the kairomone presented in this thesis, further chemical analyses are needed to fully identify the structure of the compounds involved. Comparative studies on the odours emanated by the other hosts of *L. distinguendus* may help to narrow down the number of chemicals that might be responsible for the attractiveness.

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