CHAPTER 10

Summary

The general objective of this thesis were chemoecological aspects concerning the mate and host finding process of parasitic Hymenoptera belonging to the family of Pteromalidae (Chalcidoidea). Most investigations were done with the solitary ectoparasitoid *Lariophagus distinguendus* attacking larvae and pupae of several beetle species, most of them pests of stored plant products. The main emphasis of this study was the chemical and functional characterisation of natural products functioning as pheromones or host-associated kairomones in the mate finding and recognition process. Furthermore, basic data on the reproductive performance of *L. distinguendus* were acquired and the influence of host-associated mould was investigated on fitness and chemical orientation of the parasitoid. In addition, comparative studies were conducted to elucidate the role of male and female pheromones in the sexual communication of the related species *Nasonia vitripennis* developing gregariously in pupae of cyclorraphous dipterans.

(1) Characterisation of the courtship pheromone in *L. distinguendus*

A previous study had demonstrated that the stereotypical courtship behaviour of *L. distinguendus* males is initiated by a sex pheromone that is active only at short distances. The chemical compounds involved should be investigated more thoroughly in this thesis. Experiments revealed that pheromone production started already during pupal development. Interestingly, not only female but also male pupae were shown to release the behaviourally active compounds. So far, it had been assumed that the courtship pheromone is female-derived. However, males metabolised the active chemicals within 32 hours after emergence whereas females remained active. In total, 72 compounds were identified by GC-MS in whole body extracts of male and female *L. distinguendus*, the majority of which were typical cuticular hydrocarbons. Major components were methyl-branched alkanes with one to four methyl groups, whereas saturated aliphatic and monounsaturated alkanes occurred as minor compounds. The chain length of alkanes varied between 25 and

37 carbon units. After fractionation of active whole body extracts by adsorption chromatography, only the nonpolar hydrocarbon fraction caused arrestment and elicited elements of the male courtship behaviour (chapter 2).

The fact that pheromone activity was present in hydrocarbon fractions of females and freshly emerged males but not in those of older males, was used to narrow down putatively bioactive components of the hydrocarbon profiles. For this purpose, relative amounts of the hydrocarbon profiles of active and inactive fractions were compared by *principal component analysis* (PCA) and *partial least squares-discriminant analysis* (PLS-DA). Active and inactive hydrocarbon profiles were clearly distinguishable and former ones were particularly characterised by a series of methyl-branched alkanes, such as 3-methylhexacosane, 3-methylheptacosane, 3-methylnonacosane, 3,7-dimethylheptacosane and 3,7-dimethylnonacosane (chapter 3).

Further investigations demonstrated that males searching for mates are able to recognise grains containing developing conspecifics but are not able to differentiate between those containing females and males. Immature males might therefore benefit from releasing the pheromone by distracting their already emerged competitors away from searching for actual females. The phenomenon was discussed with respect to a novel case of pre-emergence chemical mimicry (chapter 2).

(2) Studies on the reproductive performance of *L. distinguendus*

With respect to the understanding of the sexual communication in parasitic Hymenoptera, further experiments on the mating characteristics and the reproductive performance of *L. distinguendus* were done. Males were shown to copulate with up to 17 out of 20 females offered successively within a 10 hour test period. The amount of sperm transferred to the females during copulation decreased significantly with increasing number of copulations of the males. Nevertheless, males continued to mate even when they transferred only small amounts or no sperm at all. Unlike males, the majority of *L. distinguendus* females mated only once. Second matings were observed only in a few cases. Thereby, the tendency of females to mate a second time did not depend on the mating status of the male with which the females had initially mated, suggesting that remating in

L. distinguendus females does not occur as a result of sperm deficiency. Furthermore, females that had copulated with a multi-mated male did not release the sex pheromone longer to maintain the chance for a second mating than females that received a full sperm load. In both cases, pheromone activity decreased with increasing time after copulation. These results were discussed with respect to a putative mating strategy of sperm-depleted males which might increase their fitness indirectly by continuing with mating to limit other males' access to receptive females (chapter 4).

(3) Characterisation of the courtship pheromone in *N. vitripennis*

A comparative study on the sex pheromone of *N. vitripennis* revealed several conformities with *L. distinguendus* but also some interesting differences. Neither female nor male *N. vitripennis* showed pheromone activity during pupal development suggesting that females produce the behaviourally active compounds not until emergence. Paper discs treated with extracts of *N. vitripennis* females arrested male parasitoids. However, more complex elements of the typical courtship behaviour were elicited only when extracts were applied on dummies (extracted male cadavers) suggesting that not only chemical but also optical and/or tactile cues play a role in the courtship of *N. vitripennis*. Fractionation of active extracts of females demonstrated that pheromone activity is located in the hydrocarbon fraction as previously shown for *L. distinguendus*. Chemical analysis by GC-MS revealed that exclusively methyl-branched, saturated and monounsaturated aliphatic alkanes with chain lengths from 25 to 37 carbon units were present in the active fraction. Most of the 67 compounds occurred both in inactive male and active female fractions. However, the relative abundances of the individual hydrocarbons were often substantially different between the sexes. Results suggest that the use of cuticular hydrocarbons as courtship pheromones is a common feature at least in the Pteromalidae but possibly also in other taxa (chapter 5).

(4) Identification of a male sex pheromone in *N. vitripennis*

While female sex pheromones have been demonstrated in many parasitoid species and also identified in a few cases, there is hardly anything known about male pheromones. Analysis of whole body extracts of male and female *N. vitripennis* by

GC-MS revealed a male-specific diastereomer mixture of (4*R*,5*R*)- and (4*R*,5*S*)-5-hydroxy-4-decanolide (HDL). The compounds were mainly found in the abdomen of the males and apparently released intermittently. Experiments in a static four-chamber olfactometer demonstrated that virgin females but not males were attracted to both the natural and the synthetic HDL-diastereomers. Thus, HDL is the first identified male-derived sex pheromone in parasitic Hymenoptera. However, the response of females to the sex pheromone was controlled by their mating status. A few minutes after copulation females avoided the HDL-mixture. A neutral response was shown by mated females after 24 hours and still after they had been allowed to oviposit for 6 days. *N. vitripennis* females normally mate only once since sperm received from a single insemination is sufficient in most cases to fertilise a lifetime supply of eggs. Thus, the variable response to the HDL depending on the mating status makes sense from an evolutionary perspective because it increases the chance of virgin females to mate and decreases the probability for mated ones to be harassed by male parasitoids when searching for oviposition sites (chapter 6).

(5) Characterisation of host-associated kairomones used for mate and host finding in *L. distinguendus*

When volatile sex pheromones are missing like in *L. distinguendus*, parasitoids have to rely on other chemical cues for long-range orientation towards their mates. One possibility for the males is to use the same host-associated odours for mate finding that females use for host finding. Olfactometer experiments demonstrated that both males and females of *L. distinguendus* were attracted to volatile compounds emitted by the larval faeces of their host *Sitophilus granarius*. After fractionation of bioactive headspace extracts of larval faeces by adsorption chromatography, both the pentane and the methanol fraction were necessary to maintain the attractiveness. Thus, the host-associated kairomone is composed of compounds with different polarities. While the composition of the polar fraction was chemically analysed by GC-MS, the structure elucidation of the nonpolar components was impossible so far since they occurred only in minute amounts. By using host-associated volatiles emitted by larval host faeces *L. distinguendus* males can be arrested in the vicinity of foraging females and thus, increase the probability of finding mating opportunities (chapter 7).

(6) Studies on the influence of mould infestation on the fitness and chemical orientation of *L. distinguendus*

Several aspects of the chemically mediated host finding process of *L. distinguendus* have been intensively investigated in recent years. However, the fitness of female parasitoids is not only characterised by the effective location of hosts and the number of eggs they lay but also by the survival and optimal development of their offspring. Thus, the ability of females to evaluate the quality of host patches and hosts is crucial to maximise their reproductive success. Intense growth of mould in host habitats of *L. distinguendus* can lead to distinct sites of extreme environmental conditions (hot spots) with increased insect mortality. Thus, the influence of mould infestation on the fitness and chemical orientation of *L. distinguendus* was investigated. Olfactometer experiments demonstrated that female parasitoids avoided volatiles released by the two storage fungi *Aspergillus sydowii* and *A. versicolor*. As it was known that larval faeces of the granary weevil *S. granarius* is used by the parasitoids for host finding, the attractiveness of larval faeces from moulded host cultures was also tested. Faeces from moulded weevil cultures remained attractive (*A. versicolor*) or caused a neutral response (*A. sydowii),* but parasitoids clearly preferred the odour of non-moulded faeces when offered simultaneously. The common fungal volatile 1-octen-3-ol was detected as a major component among the volatiles of larval faeces from moulded host cultures and repelled female parasitoids in the olfactometer at natural concentrations. *L. distinguendus* had a clearly reduced fitness when hosts from moulded weevil cultures were offered for oviposition. These results suggest that *L. distinguendus* females use 1-octen-3-ol for host habitat assessment to avoid negative fitness consequences due to secondary mould infestation of host patches. The fact that the response of females to the fungal volatile is innate, indicates that fungi played a crucial role in the evolution of host finding strategies of *L. distinguendus* (chapter 8).

Finally, main results and conclusions of this thesis were discussed in a broader chemoecological context. A complex and fascinating network of infochemicals mediating mate and host finding of *L. distinguendus* was presented by combining the findings of this work with those of previously published studies. Furthermore, directions for future studies were given (chapter 9).

The identification of natural compounds used by parasitic Hymenoptera for sexual purposes represents an almost unexplored field of chemical ecology. Thus, results of this thesis provide new insights into the sexual communication of these ecologically and economically important insects. The combined use of behavioural, chemical and statistical methods as presented here might be exemplary for further studies on infochemicals used by parasitoids.