Ecology of *Drosophila* aggregation pheromone: a multitrophic approach

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Ecology of *Drosophila* aggregation pheromone: a multitrophic approach

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. dr. ir. L. Speelman
in het openbaar te verdedigen
op maandag 1 oktober 2001
des namiddags te half twee in de Aula.

Wertheim, Bregje (2001).
Ecology of *Drosophila* aggregation pheromone: a multitrophic approach

Thesis Wageningen University. -with ref. -with summary in Dutch

ISBN: 90-5808-433-7

Printed by Ponsen & Looijen bv, Wageningen, the Netherlands.

STELLINGEN

1. Elk voordeel heb z'n nadeel.
   J. Cruijff, dit proefschrift

2. Micro-organismen die bij de kolonisatie van nieuwe habitats beperkt worden in hun reproductief succes door lage populatiedichtheden kunnen baat hebben bij het exploiteren van een vector met aggregatiegedrag.

3. Het is waarschijnlijk dat een mutualistische interactie tussen micro-organismen en insecten meerdere malen heeft geleid tot de evolutie van aggregatieferomonen. De veronderstelling dat aggregatieferomonen slechts 'mannelijk geproduceerde sexferomonen' zijn is onaannemelijk.
   contra Landolt (1997) American Entomologist, 43: 12 - 22, dit proefschrift

4. Populatiedynamica wordt wel degelijk in belangrijke mate beïnvloed door variatie en flexibiliteit in individueel gedrag.
   contra Ives et al. (1999) The American Naturalist, 154: 652 - 673, dit proefschrift

5. Ieder voedselweb gaat vergezeld van een informatieweb en dat heeft grote gevolgen voor multitrofe interacties.
   dit proefschrift

6. De kosten-batenbalans van het verbod van de rooms katholieke kerk op condoomgebruik zal negatief uitvallen: het aantal geboorten door het verbod op condoomgebruik zal niet opwegen tegen de sterfte als gevolg van dit verbod.

7. De vrijhandel blijft een farce zolang de rijke landen hun eigen exportmarkt beschermen met exorbitant hoge invoerrechten op alles behalve primaire grondstoffen.

8. Als de maatschappij een beroep doet op wetenschappelijke expertise moet niet de mening van één wetenschapper de basis vormen voor de maatschappelijke opinievorming maar moet juist de discussie tussen wetenschappers centraal staan, omdat de belangrijkste informatie in de nuances zit.

de Avond van Wetenschap en Maatschappij, 6 november 2000

9. Het is wrang dat de personeeltekorten bij de overheid voor een belangrijk deel veroorzaakt worden door de uitstroom van gemotiveerde, goed opgeleide werknemers die een gemis ervaren aan toekomstperspectief, terwijl de specifieke overheidstaken – zoals gezondheidszorg, onderwijs en fundamenteel onderzoek – bij uitstek duurzaam zijn.

10. De vergrijzing binnen de Wageningen Universiteit neemt zorgwekkende vormen aan, hetgeen blijkt uit het verschijnen van advertenties van begrafenisondernemers in het universiteitsblad Wb.

11. There is always one more bug.

Murphy's software programming law

12. Het gemier over muggen en vliegen draagt bij aan de overlast.

13. Voor wandelvakanties geldt: alles wat je thuislaat is mooi meegenomen.

Stellingen behorend bij het proefschrift van Bregje Wertheim
'Ecology of *Drosophila* aggregation pheromone: a multitrophic approach'
Wageningen, 1 oktober 2001

CONTENTS

ABSTRACT

Many insect species use an aggregation pheromone to form groups with conspecifics in certain localities of the environment. This type of behaviour has a variety of implications for ecological interactions, both directly through the effect of the pheromone on the behaviour of con- and heterospecifics, and indirectly through the consequential aggregative distributions that may affect species interactions. The evolutionary ecology of the use of aggregation pheromone has received only little attention. Yet, these pheromones may play an intricate role in food web interactions by providing an accompanying information web.

The aim of this thesis is to further our understanding on the ecological and evolutionary aspects of the use of aggregation pheromone in insects. By unravelling costs and benefits that arise from the use of aggregation pheromone in our ecological model organism, *Drosophila melanogaster*, we strive to answer *why* they use an aggregation pheromone and elucidate the ecological consequences of an aggregation pheromone in a food web context.

In laboratory and field studies, we identified behaviours and interactions of the fruit fly *D. melanogaster* that were affected by its aggregation pheromone. The pheromone affected the distribution of adults, their eggs, competitor species and parasitoids. Moreover, a number of costs and benefits to the use of aggregation pheromone were indicated. In subsequent studies, the major hypotheses on costs and benefits were examined.

A major benefit of using aggregation pheromone was shown to be aggregated oviposition. Aggregated oviposition enhanced the quality of the larval resource, as indicated by a higher survival of the larvae and larger size of the emerging flies. This Allee effect was characterised by a positive effect of adult density on larval fitness components, and may have arisen from the interaction between adult flies and micro-organisms (yeasts and fungi). Fungi antagonise yeast and larval development, while adults can inoculate yeast on a substrate and temper fungal growth. Larvae also tempered fungal growth, but an increased larval density did not result in an Allee effect but in competition instead.

A major cost of using aggregation pheromone arose from an increased risk of parasitism. The parasitoid *Leptopilina heterotoma* uses the aggregation pheromone of adult fruit flies to localise the larval hosts, and based on this information this parasitoid can differentiate quantitatively at long range between substrates that differ in profitability. After arrival on a substrate, the pheromones no longer play a role in the host searching behaviour. A behaviour-based model was developed to predict the individual risk of parasitism for hosts in differently sized host aggregations. The functional and numerical responses of the parasitoids were combined with a flexible patch leaving decision rule for the parasitoid, to assess whether aggregation could also comprise a benefit to the hosts in terms of a diluted risk (*sensu* Hamilton 1971). The model prediction reads that aggregation is not beneficial in the context of the *Drosophila* - *Leptopilina* interaction, and these predictions were supported by field data.

In a simple spatio-temporal simulation model, the population dynamics arising from several modes of dispersal, food competition and an Allee effect were explored. The model is a first step towards a more extensive model that incorporates the responses of insects to spatially heterogeneous resources and chemical information (e.g., aggregation pheromone).

The main conclusion from this thesis is that the aggregation pheromone of *D. melanogaster* plays an intricate role within a foodweb context, and that a variety of costs and benefits arise from multitrophic interactions. To understand the dynamic interactions in this and many other ecological systems, it is essential to gain more insight into the effect of aggregation pheromone on the behaviour of individuals.

CHAPTER 1

General introduction and summary

Evolutionary ecology

The distribution of individuals across the environment is largely determined by biotic interactions and environmental characteristics (Begon et al. 1996). These features affect survival probabilities and reproductive output in each locality. Natural selection has shaped the phenotype of organisms to deal optimally with the conditions and interactions that they may encounter in the environment. This is the theme of evolutionary ecology.

Selection acts at the interplay between individuals and their environment. The environment of individuals comprises many other individuals of various species, and the interactions with these organisms can shape individual morphology, physiology and behaviour. Moreover, the environment is a heterogeneous patchwork, that exhibits large variation in both space and time, setting the stage for the evolution of an incredible diversity of strategies in ecological interactions. These strategies are founded on selfishness: a strategy is successful when it provides a gain to the individual, also when alternative strategies would be better for the common good.

Animals can behaviourally respond to heterogeneity in localities, and opt for the most favourable localities that are available to them. Profitability of a locality is delimited by, for example, the quantity and quality of food sources, the risk of attack by natural enemies, the density of conspecifics and competitor species, and the availability of refugia that provide shelter from harsh conditions. Individuals may use cues to estimate habitat profitability and adjust their behaviour accordingly (e.g., Wagner and Kurina 1997, Turner et al. 2000, Prokopy and Roitberg 2001). However, concessions are inevitable, the cues are neither infallible nor comprehensive, and profitability is not static but changing over time, also under the influence of the numbers and characteristics of other organisms. Furthermore, what appears best in one locality, might prove a poor strategy in others. Hence, the strategy of an individual is likely to result in both costs and benefits that vary for different environments.

When animals respond to the presence of conspecifics when selecting a locality, a range of spatial distributions can arise as a result (fig. 1). The topic of my thesis is the evolutionary ecology of an aggregated distribution of animals across the environment (fig. 1), where the animals respond behaviourally to the presence of conspecifics and actively seek out each other. Both the aggregated distribution and the responsiveness to conspecifics affect a variety of ecological interactions (Parrish and Edelstein-Keshet 1999). Moreover, a number of disadvantages for this type of behaviour are apparent, and yet, the behaviour persists. This raises the questions: Why do these animals aggregate, how has this evolved and what are the ecological implications for the individual, the population and the community?

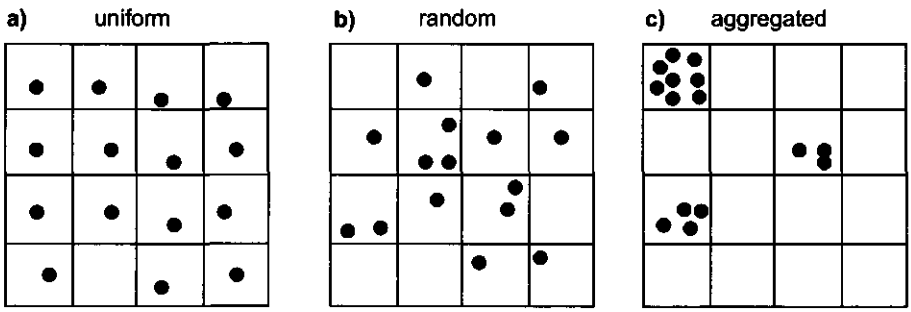

Figure 1: Schematic drawings on spatial distributions of organisms. The large squares represent the whole environment, that comprise localities (the compartments) that are assumed to be equal in all characteristics, apart from the number of individuals (the dots). a) When individuals avoid each other, the resulting spatial distribution is uniform or spaced-out. b) When individuals choose a locality, regardless of the presence of other individuals, the resulting spatial distribution is random. c) When individuals seek out each other, the resulting distribution is aggregated or clustered.

Costs and benefits

When we ask 'why' an organism exhibits a certain trait, we are interested in the way that the trait contributes to the fitness of the individual, irrespective of whether the trait concerns morphology, physiology or behaviour. Fitness is a relative measure that describes the number of descendants that an individual leaves, relative to other individuals that exhibit alternative traits. As stated above, traits evoke both costs and benefits to the bearer. The evolution and persistence of a particular trait requires that benefits outweigh the costs.

Costs and benefits must be expressed in a currency, that captures the crucial component of fitness (Krebs and Davies 1993). Examples are the rate of offspring development, the number or size of the offspring, the survival probability throughout a day, or the numbers of mates in a lifetime. Specifying a currency is based on a hypothesis. The hypothesis is put to the test by formulating predictions on the trait, and subsequent experiments will provide evidence that is in favour or against the hypothesis. Usually, a variety of interactions affect the costs and benefits of a trait, and ideally all should be scrutinized to result in a conclusive appraisal (Dicke and Sabelis 1992). In reality, the complexity of ecological webs is beyond what can feasibly be investigated, and a selection is made to include only the main interactions.

An underlying assumption of studying costs and benefits is that the individual will do what is best for him/her, given the constraints that apply (e.g., environmental, genetical and physiological constraints). The assumption of 'doing the right thing' is based on the conviction that natural selection has optimized the individual to the demands of the environment. This assumption is not being tested, but accepted a priori. Undoubtedly, this may not always be justified, and caution is warranted while interpreting the data on cost-benefits (Godfray 1994). However, the approach of analysing costs and benefits is especially

suitable for experiments, and with caution taken, it may yield valuable insight in the processes that shape the traits of individuals.

For the aggregated distributions that are central to my thesis, a variety of costs and benefits may apply (Pulliam and Caraco 1984, see also chapter 2). Additionally, the cues that are involved in attracting conspecifics can markedly affect the interactions with both conspecifics and heterospecifics. The basic assumption is that forming aggregations optimizes the fitness of the participating individuals. My question is, why is this so? Which benefits promote aggregative behaviour, and at what costs?

The ecological significance of aggregative behaviour

Aggregative behaviour has large implications for the ecology of animals. Animals in a group interact with group members to a distinctively larger degree than with other individuals. This grouping affects their behaviour (e.g., time allocation to feeding, contests or parental investment), their morphology (e.g., size, dominance characteristics) and physiology (e.g., hormonal titre). Ultimately, aggregation influences their survival probabilities and their reproductive success. At the population level, aggregative distributions of individuals influence the degree of competition between conspecifics and heterospecifics, dispersal, and the interactions with natural enemies. Aggregative distributions create heterogeneity in the strength of interactions between species. Whereas in some localities a species might be dominated by others, at other localities the dominant species might be absent, or its superiority might be constrained. This may facilitate stable coexistence of species, either competitor species or natural enemies and their victims, and hence promote biodiversity. Conversely, aggregative distributions can lead to over-exploitation of a resource, rapid spread of infective diseases and consequentially, to massive extinctions. To understand, predict and manage the dynamics in ecological systems, it is essential to gain more insight into the role of aggregative behaviours of individuals.

Information conveyance and communication

The aggregative behaviour that is discussed here arises from responses of individuals to the presence of others. This implies that individuals release information on their whereabouts, either inevitably by their actions or deliberately, and that others pick up on this and adjust their behaviour in response. The cues that are involved in such information conveyance can be visual, chemical, auditory or tactile. The cues that are central to this thesis are aggregation pheromones. Aggregation pheromones are chemical substances, released by an individual, that attract and/or arrest conspecifics at the locality of the sender. When both the responder and the sender benefit from the information conveyance, it is called communication (Bradbury and Vehrencamp 1998). However, many alternative forms of information conveyance occur. The cues that are released by an individual can be exploited by everyone in the food web (Haynes and Birch 1985, Vet and Dicke 1992, Stowe et al. 1995, Dicke and Vet 1999, Dicke and van Loon 2000). For example, natural enemies can home in on the cues to localise their victims and competitors can eavesdrop and adjust their strategy in response to the information (e.g., avoid or overtake resources). In essence, the food web is accompanied by an information web, that may profoundly alter the ecological interactions. These information webs exist in any ecological system, and yet, their contribution

to ecological processes is poorly understood. The information web affects the dispersal and spread of organisms, the distribution of natural enemies and competitor species, and consequently, population dynamics of all food web species.

Drosophila as an ecological model organism

To investigate the complex issue of costs and benefits of the use of aggregation pheromone with respect to ecological interactions, a model system is required for which the information web can be manipulated, and the food web interactions can be studied in the laboratory and the field. The fruit fly *Drosophila* is an ideal model organism for these investigations. Many fruit flies possess aggregation pheromones that have been chemically identified and can easily be applied in experimental set-ups. These insects are common in nature, their basic food web structure and ecology is known and they are easy to culture and to work with in the laboratory and the field.

The focal species, *Drosophila melanogaster*, forms aggregations on fermenting fruit, using an aggregation pheromone. In these aggregations, they feed, mate and oviposit (Spieth 1974). The larvae and adults feed especially on the yeasts and bacteria that develop on the fruit. The larvae have an aggregated distribution across resources, as a result of aggregation pheromone use in the adults. Within resources, the larvae frequently experience (severe) competition for food. The aggregation pheromone of *Drosophila* is fairly species-specific, and other *Drosophila* species that co-occur in an environment, may use the aggregation pheromone of conspecifics and heterospecifics in selecting or avoiding breeding substrates. The most important natural enemies of the fruit fly are larval parasitoids. These parasitoids breed in a drosophilid larva and the fruit fly larva is killed when it is consumed by the parasitoid's offspring. To localise the drosophilid larvae, the parasitoid spies on the communication of adult fruit flies (Wiskerke et al. 1993a, Hedlund et al. 1996a). The use of aggregation pheromone by the adult fruit flies results in an increased conspicuousness of their larvae to natural enemies.

Thus, obvious costs may arise from the use of aggregation pheromone by adult fruit flies: The larvae experience increased competition for food and increased conspicuousness to natural enemies. Then, why do drosophilid flies use an aggregation pheromone? What benefits are sufficient to exceed these costs? Does it involve mate finding in adults, survival and growth of offspring, or a combination of factors (fig. 2)?

AIM AND OUTLINE OF THE THESIS

The aim of this thesis is to further our understanding on the ecological and evolutionary aspects of the use of aggregation pheromones in insects. As explained above, these aggregation pheromones may have a large impact on a variety of ecological interactions by providing an information web and inducing an aggregative distribution. By unravelling costs and benefits that arise from the use of aggregation pheromone in *Drosophila melanogaster*, ecological consequences of an information web for food web interactions are elucidated. The main ecological interactions that are affected were selected in a field study (chapter 3), and the investigations on costs and benefits have been restricted to these.

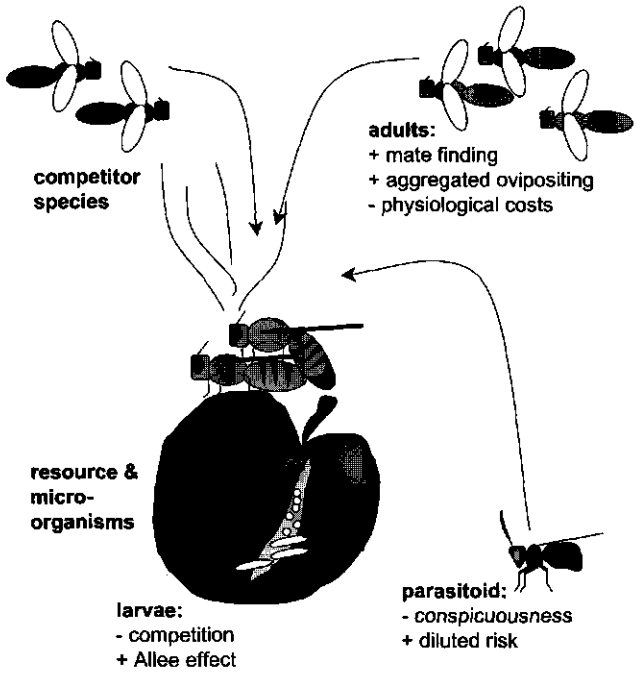

Figure 2: The information and food web interactions in the *Drosophila* system, and the potential costs (-) and benefits (+) for *D. melanogaster* that arise from the use of aggregation pheromone. Adult males and mated females release the pheromone when situated on a substrate (fruit). The pheromone attracts conspecific adult flies, that rather select a substrate containing the aggregation pheromone than a substrate without such fruit fly odours (see also fig. 1). In the adult aggregations that arise, the flies feed, mate and oviposit. Consequently, larvae are also aggregated on those (few) substrates that contained fruit flies (see also fig. 1). The larvae feed on the yeasts that grow on the resource. When larval densities are high, the larvae compete for food. When larval densities are low, larvae may be unable to cultivate the fruit such as to enhance the growth of yeasts and reduce fungal contamination (i.e., the Allee effect). Not only members of their own species are attracted by the use of aggregation pheromone, but competitor species and parasitoids as well. The competitor species also oviposit on the resource, and their larvae could contribute to the cultivation of the resource (which comprises a mutual benefit), but may also increase the larval competition for food. The parasitoid spies on the communication of adult flies, and because of the pheromone, the larvae are more conspicuous to their natural enemy. On the other hand, aggregation may also dilute the risk of attack for the larvae. When the parasitoid attacks only a limited number of all the larvae that are present, the chance of attack per individual decreases when the number of larvae increases.

Chapter 2: The occurrence, benefits and costs of the use of aggregation pheromone in insects is reviewed. Aggregation pheromones are reported in over 250 non-social insect species, belonging to 35 different families in 10 different orders. Thus, the use of aggregation pheromone is widespread among insects. The mechanisms for signal transmission are highly variable, and these have been studied in great detail. The benefits of aggregation pheromone use have received relatively little attention in the literature, but the benefits for most species can be tentatively assigned to one or more of the following categories:

1) increased efficiency in resource exploitation; 2) mate finding; 3) protection from natural enemies; 4) protection from environmental conditions; or 5) aggregated oviposition. A number of costs is also discussed. The survey revealed striking similarities within taxa (indicating phylogenetic predisposition) and among taxa (indicating convergence). The emphasis in most studies on aggregation pheromone is on mechanisms, chemical identity of the pheromone and applications for pest management. The shortage of integrated studies on ecological, functional and evolutionary aspects of the use of aggregation pheromone severely hampers our perception of the ecological significance of this communication behaviour. The remainder of this thesis specifically addresses these aspects for aggregation pheromone in *Drosophila*.

Chapter 3: A field study was conducted to explore the variety of behaviours and ecological interactions that are affected by the use of aggregation pheromone in *Drosophila melanogaster* and to assess the magnitude of these effects within the full web of ecological interactions. The role of aggregation pheromone in the selection of resources, oviposition site selection, behaviour on a resource and interspecific interactions was investigated. The pheromone comprises both direct effects (spatial distribution of adults, eggs, competitor species and natural enemies) and indirect effects (increased interference among adults, increased competition among larvae). Both types of effect can yield costs and benefits to the fruit flies. The information and food web of *Drosophila* are revealed and the main interactions that are of importance are identified (fig. 2). These will be presented more thoroughly in the remainder of the thesis.

Chapter 4: Behavioural plasticity enables an individual to adjust and optimize its strategy to various conditions, in response to an altered cost-benefit balance. To explore costs and benefits that are associated with the use of aggregation pheromone, some aspects of behavioural plasticity in adult *D. melanogaster* were evaluated in laboratory bio-assays. Based on the field experiments (chapter 3), two hypotheses were formulated on a benefit concerning aggregated oviposition. One hypothesis concerns a benefit to the adult females, where reduced harassment by males can enhance the oviposition rate; the other concerns a benefit to their offspring, where groups of larvae can exploit arduous resources more efficiently. The response of the adults to the aggregation pheromone was significantly weaker for high quality substrates. This was in support of the larval resource exploitation hypothesis. It may indicate that aggregation of offspring facilitates the larval exploitation of demanding resources, whereas in high quality substrates, aggregation is less necessary.

Chapter 5: The evolution of an aggregation pheromone requires that individuals benefit from clustering. Such a situation can arise when survival or reproduction is hampered at low population density, i.e., with an Allee effect. The hypothesis that aggregated oviposition in females yields a benefit for larval development was tested. To identify positive effects of aggregation (i.e., an Allee effect) on the survival and growth of *D. melanogaster* larvae, larvae were incubated at different densities and their development was monitored. A potential effect of the presence of adult flies was also investigated by incubating a varying number of adults on the substrate before introducing the larvae. Increased larval density resulted only in food competition, but increased adult density prior to larval introduction enhanced survival and

growth of the larvae. This substantiates our hypothesis on a significant benefit for aggregated oviposition in fruit flies, through enhanced larval development. The pattern may be attributed to the interaction between adults, micro-organisms (yeasts and fungi) and larvae. The larvae of *D. melanogaster* feed on yeasts that develop on fruit, but fungal growth antagonises yeast and larval development. Adult flies inoculate fruits with yeast and reduce fungal growth. Hence, increasing adult densities enhanced the quality of the substrate for larval development.

Chapter 6: The larval parasitoid *Leptopilina heterotoma* spies on the aggregation pheromones of adult fruit flies. The female parasitoid is attracted to substrates with aggregation pheromone and this helps her to localise her larval hosts. The effect of the fruit flies' aggregation pheromone on different aspects of host-searching behaviour of the parasitoid was investigated in laboratory and field experiment. The results show that with her behavioural responses to aggregation pheromone, the female parasitoid quantitatively differentiates at long range between patches that differ in profitability, thereby reducing a waste of time in non-profitable habitats. After arrival on a substrate, the aggregation pheromone no longer plays a role in the searching behaviour for hosts, and the parasitoid uses mainly other cues to decide where and for how long to search on the substrate. In several field experiments, parasitization was higher in substrates with aggregation pheromone than in control substrates without aggregation pheromone. This reveals an ecological cost to the use of aggregation pheromone in adult *D. melanogaster*, in terms of an increased risk of parasitism for their offspring.

Chapter 7: Parasitoids can exploit aggregation pheromones for localising hosts, but simultaneously, hosts may experience a lower individual attack rate from natural enemies when they form aggregations and 'shield in a herd'. The latter phenomenon has frequently been used as a functional explanation for aggregative behaviour. The decreased efficiency of parasitoids arises, for example, through the waste of time in (re)encounters with already parasitized hosts, or because more hosts are present than can be a exploited by a parasitoid. However, this could be opposed by a variety of flexible behavioural responses of the parasitoid to the density of the hosts. To predict the individual risk of attack for hosts in aggregations, one has to combine the effects of higher parasitoid loads on aggregations (in response to the aggregation pheromone), the reduced searching efficiency of parasitoids in such aggregations, and the behavioural flexibility of the parasitoid in response to host density. To assess whether and when aggregation is beneficial for individual hosts with respect to risk of parasitism, we developed a simple mathematical model, based on parasitoid behaviour. The model prediction is that aggregation is not beneficial to *Drosophila* in the context of the *Leptopilina* - *Drosophila* interaction, and the use of aggregation pheromone augments the individual risk of parasitism across all host densities. The results of a field experiment were in qualitative agreement with this prediction: the risk of parasitism for individual hosts increased with increasing host densities. Thus, the use of aggregation pheromones constitutes an ecological cost with respect to the risk of parasitism, and the benefits of aggregative behaviour itself have to be sought in other directions than escape from natural enemies.

Chapter 8: The use of aggregation pheromone implies a spatial process. It affects the dispersal of individuals and creates heterogeneity in local densities. With a spatio-temporal simulation model, the dynamics arising from several modes of dispersal, food competition and the Allee effect are explored. The model is loosely based on *Drosophila*, but kept as simple as possible. The model predicts that the establishment and persistence of a fruit fly population is determined by the initial distribution of adult flies, the availability of resources, the ability to reach resources and spatial heterogeneity. The model is a first step towards a more extensive model that incorporates the responses of insects to spatial heterogeneous resources and chemical information (e.g., aggregation pheromone).

Chapter 9: This chapter is a synthesis of all previous chapters on the ecological costs and benefits that are associated with the use of aggregation pheromone in *D. melanogaster*. The evolutionary origin and ecological implications of the use of aggregation pheromone are discussed, and directions for future investigations are suggested. My main conclusion from this thesis is that aggregation pheromones play an intricate role within a food web context, and a variety of costs and benefits arise through their direct and indirect influences on ecological interactions. The costs and benefits for the use of aggregation pheromone are different for male and female fruit flies and depend largely on the characteristics of the environment. Whether fruit flies should emit the pheromone and respond to it depends to a large extent on the density and quality of substrates and the risk of attracting parasitoids and competitors. To bridge the gap between individual behaviour and spatial population dynamics, it is essential to integrate the causal mechanisms of behaviour, the function of the behaviour for the individual and the ecological implications for food web interactions.

CHAPTER 2

Aggregation pheromones in non-social insects: An ecological and evolutionary perspective

Abstract

Aggregation pheromones have been recorded in numerous non-social insect taxa. The evolutionary and ecological aspects of aggregation pheromones, however, have received little attention. Why do some insects use aggregation pheromones? And is the evolution predisposed by phylogeny or certain ecological characteristics? This review compares the mechanisms and functions of aggregation pheromones that have been recorded across 250 different insect species, belonging to 35 families in 10 orders. The behavioural and physiological mechanisms are summarized for all taxa and possible functions for both responders and emitters are deduced. Most species are assigned to one or more of the following categories: 1) efficient resource exploitation, 2) mate finding, 3) protection from natural enemies, 4) protection from environmental conditions and 5) aggregated oviposition. Similarities within and across taxa are described and linked to the ecology of the insects. Within taxa, large resemblances were reported for both mechanism and function. One striking similarity across taxa is the association with fungi and micro-organisms amongst aggregation pheromone-possessing insects. On the other hand, predation and haematophagy as well as feeding on healthy plants is relatively uncommon among insects with aggregation pheromones. Subsequently, ecological costs of the use of aggregation pheromones are reviewed and the role of aggregation pheromones in ecological interactions with competitor species and natural enemies is discussed. By comparing the different insect orders, general patterns emerge that can guide further evolutionary and ecological investigations.

Introduction

Animal aggregations are a general phenomenon in ecological systems that affect many spatial and temporal processes (Parrish and Edelstein-Keshet 1999). An important mechanism leading to the formation of animal aggregations is communication between individuals. Through communication, they actively seek out each other and form assemblies for certain periods of time. Several modes of communication exist (e.g., auditory, visual, chemical), and each has its own advantages and disadvantages, such as reach, directionality, speed and specificity (Bradbury and Vehrencamp 1998). Depending on the environment, the ecology and the physiology of the organisms, a certain set of signals evolves (Baker 1985, Bradbury and Vehrencamp 1998).

In insects, as in most other animals, chemical information conveyance is of profound importance (Bell and Cardé 1984, Baker 1985, Roitberg and Isman 1992, Cardé and Bell 1995, Cardé and Minks 1997). The signals in chemical information conveyance (info- or semiochemicals) that induce group formation are termed aggregation pheromones. Signals in information conveyance can mediate both intra- and interspecific interactions, and have therefore a major impact on different ecological research areas, such as behavioural ecology, evolutionary ecology, population dynamics, predator-prey interactions and community ecology. Even though this is recognized, studies on aggregation pheromones are seldomly integrated into functional ecological research, and they are mostly confined to mechanistic and applied approaches. The specialized organs for production and perception of aggregation pheromones and the chemical composition of pheromones have been studied in great detail (e.g., Borden 1985, Byers 1991). In addition, the applications of aggregation pheromone for pest management are being explored (e.g., Vite and Baader 1990, Howse et al. 1998). In contrast to this emphasis on mechanistic and applied research, relatively little attention has been directed to the evolutionary and ecological aspects of aggregation pheromones: Why do some insects use aggregation pheromones? What are the advantages for both emitter and responder? Is the evolution of aggregation pheromones predisposed by phylogeny or certain ecological characteristics? What consequences do aggregation pheromones have at higher level processes, such as population dynamics and community ecology? The few and mostly older reviews that have incorporated these topics were restricted to single taxonomic groups (Verhoef et al. 1977, Raffa and Berryman 1983, Aldrich et al. 1984, Lockwood and Story 1986), or to groups defined by one specific aspect of their ecology (McCall and Cameron 1995, Landolt 1997).

In this review, we address the literature on aggregation pheromones in insects from an evolutionary and ecological perspective, and we elucidate the magnitude of effects they have on the individual, population and community level (fig. 1). We start with specifying the boundaries of the definition of an aggregation pheromone, and present some general ideas on why insects use them. Then we shortly describe the diversity in mechanisms that is found in different insect species. By integrating chemical, physiological, ecological and behavioural studies, we infer the impact that the chemical has on the individual insect in its environment. Using comparisons across a large number of species and families, we suggest possible benefits for the individuals in aggregations. Subsequently, we discuss the costs of the use of aggregation

pheromones and the role of aggregation pheromones in interspecific interactions. Finally, we will identify important gaps in our understanding.

Aggregation pheromones: a definition

Pheromone

A pheromone is defined as an infochemical that mediates an interaction between conspecifics in which the benefit is to the sender, to the receiver or to both (Dicke and Sabelis 1988). The pheromones can originate from specialized secretory glands or from body orifices and organs involved in digestion and reproduction (e.g., mouth, anus, penis). The emission of pheromones can be controlled by the sender, be ungovernable or continuous. The perception of pheromones involves often advanced chemosensory organs, that in insects are mostly located on the antennae and on the tarsi or mouth appendages. After emission of an olfactory stimulus, a spatial gradient arises with highest concentrations at the source, the sender. This gradient can be used by receivers to locate the source of the pheromone (Shorey 1973). The communication by chemicals is slow, and after emission no longer under control of the emitter, but specificity and richness is high and reach or 'active space' can be very large (Baker 1985).

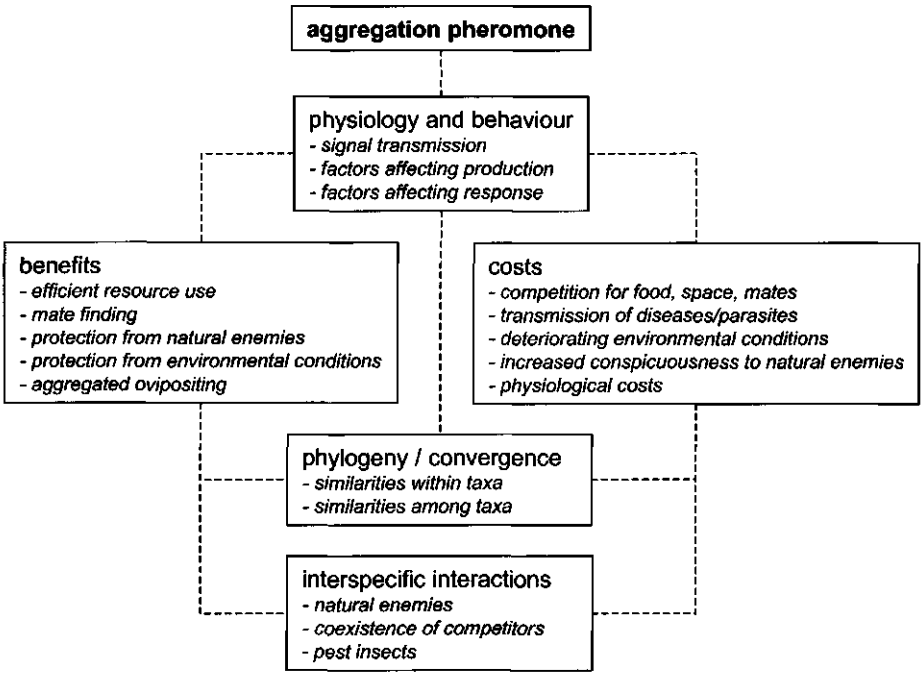

Figure 1: Major components that determine the ecology and evolution of aggregation pheromones, as discussed in the paper.

Perception of a pheromone can cause an immediate behavioural response (releaser effect) or induce a set of physiological changes (primer effect) (Nordlund and Lewis 1976). Releaser pheromones are often categorized by their function and the behaviour they induce, e.g. sex pheromones, alarm pheromones, trail pheromones and aggregation pheromones (e.g., Birch 1974, Bell and Cardé 1984, Baker 1985, Roitberg and Isman 1992, Cardé and Bell 1995). This classification is not absolute, since the same compounds can serve multiple functions (e.g., alarm ánd defence, dispersal ór aggregation depending on concentration, sexual ánd defensive), and pheromones are used in a parsimonious way by insects (Birch 1974, Blum 1996). Moreover, pheromones act often synergistically with other olfactory stimuli, meaning that their effect is greatly enhanced or even only apparent if the pheromone is perceived in combination with another chemical cue ('co-attractant') (Haynes and Birch 1985).

Aggregation pheromone

Aggregation pheromones attract and/or arrest conspecifics to the locality of release. Several more explicit definitions have been proposed, differing in designation of the sex of emitters and responders (Shorey 1973, Borden 1985, McCall 1995, Howse et al. 1998). The term aggregation pheromone is in fact only descriptive on the behaviour it induces, not on any function, contrary to most other pheromones. The function of aggregation pheromones is defined as broad as 'forming an aggregation that serves for protection, reproduction, feeding or a combination thereof' (Borden 1985). This is so general that it in practice includes every fundamental aspect of life. Landolt (1997) on the contrary states that many aggregation pheromones are in fact male-produced sex attractants, which is a very narrow interpretation of the benefits of aggregation. In our view the ecological significance of group forming by pheromones is much wider than purely sexual and not critically dependent on the exact sex of emitter or responder. We therefore decided to use a slight modification of Shorey's (1973) definition of aggregation pheromones: 'Released compound(s) causing aggregative behaviour in conspecifics of both sexes or in the same sex as the emitter'. This includes the pheromones that act within sexes, but excludes the sex pheromones of one sex that attract responders of the other sex.

Aggregation pheromones: physiology and behavioural responses

A simple query for 'aggregation* and pheromon*' in Cab-abstracts from 1972-1995 resulted in almost 1000 hits, and these articles form the material for our review. The list is likely to be incomplete, since what we included in our definition of aggregation pheromones might be called sex or oviposition pheromones by others. The studies on aggregation pheromones have widely divergent approaches, focussing on different factors influencing production and response, using field or laboratory set-ups, and applying different criteria and study aims.

In our survey, over 250 non-social species were reported to possess aggregation pheromones, belonging to 35 families in 10 different orders. We do not include social insects in our survey, since colony members are so interdependent that the principle of group living has a totally different meaning than in

non-social insects. In over 200 species, the chemical composition of the pheromones is known. Often there is a considerable overlap in the chemicals that are found in different species of the same family or genus.

Our aim for this section is to describe the diversity and similarities in mechanisms that are involved in communication by aggregation pheromones. Table 1 shortly summarizes per order and family what has been reported on the physiological and behavioural aspects of the aggregation pheromones, and this table is accompanied below by a short description on ecological aspects.

Coleoptera

Aggregation pheromones are reported for 10 families, and suggested in one other (Coccinellidae). In the vast majority of these beetle species, the pheromone is male-produced and attracts both males and females. Larvae are seldomly tested although sometimes reported to be part of natural aggregations (Bostrichidae, Cucujidae: Howe 1943, Sinha and Wallace 1966). In almost all coleopteran species, the production of and response to pheromones is critically dependent on the presence of food and food odours respectively. The aggregation pheromones are mostly emitted with faeces or originate from the hindgut. Mating often has no effect on the responses, but sometimes it causes a reduced production or production is reduced at times when females are not sexually active (Curculionidae: Booth et al. 1983). Individuals differ considerably in the amount of pheromone they produce, both genetically (Scolytidae: Schlyter and Birgersson 1989, Curculionidae: McCoy and Wright 1990) and due to plastic behaviour, such as by reducing the emission in the presence of other males, or following diurnal rhythms (Tenebrionidae: O'Ceallachain and Ryan 1977). The sex ratio of the responders can be influenced by the ratio of the different compounds in the pheromone blend, that selectively attract males and females (Cucujidae: White and Chambers 1989, Curculionidae: Ramirez-Lucas et al. 1996). Geographical variation in compound ratios is also found (Tenebrionidae: Boake and Wade 1984, Suzuki et al. 1984).

The beetles that form aggregations on feeding sites comprise stored product pest species (Bostrichidae, Cucujidae, Dermestidae, Tenebrionidae), phytophagous (Chrysomelidae, Curculionidae, Scarabidae), frugivorous (Nitulidae, Scarabidae) and wood boring species, attacking living, recently dead or decomposing trees. (Platypodidae, Scolytidae). A number of these species are vectors for host-pathogens or bio-degrading micro-organisms (Curculionidae, Nitulidae, Platypodidae, Scolytidae).

Table 1 (continued across 4 pages): Summary on the physiological and behavioural aspects of the aggregation pheromones in non-social insect taxa. For each family, the following characteristics are given: 1) Taxa, with the genera for which aggregation pheromones are reported, 2) Signal transmission, with the sexes of emitters and responders, the origin of the aggregation pheromone and the occurrence of interspecific attraction (i.e. cross attraction), 3) Factors affecting emission, 4) Factors affecting response and 5) References on which the information is based. The information per family concern at least one species in that family, but is not necessarily true for all species in that family, since mostly not all information is available. The information from contrasting reports, mostly concerning different species in the same family, are separated by a slash (e.g., + / 0). See footnote for description of signs and abbreviations.

Taxa		Signal transmission				Factors affecting emission						Factors affecting response							Refs
order and family	genera (number of species per genus)	emitter	respon- der	origin	cross attraction	food	age	matu- rity	mating	females present	males present	co- attractant	age	matu- rity	mating	crow- ding	dose	other	
Coleoptera																			
Bostrichidae	*Prostephanus (1), Rhyzopertha (1)*	m	f+m			++	0		0	-/0	-	synthetic: 0	-/0	-	0/-	+	+	food present: -	1-6
Chrysomelidae	*Phyllotreta (1)*		f+m	a.o. frass		++													7
Coccinellidae	*Hippodamia (1)*		f+m															temperatu- re: <20°C	8
Cucujidae	*Cathartus (1), Ahasverus (1), Cryptolestes (3), Oryzaephilus (2)*	m	f+m	frass	+	++	0	+			-/0	fungus- derived volatiles	+	-/0	m+/ f-	-	0	starvation: + low density population: +	9-16
Curculionidae	*Anthonomus (2), Conotrachelus (1), Cosmopilites (1), Hylobius (1), Metamasius (1), Phlyctinus (1), Pissodes (3), Rhynchophorus (4), Sitona (1), Sitophilus (3), Smicronyx (1)*	m (18) /f+m (1)	f+m	frass / hindgut	+	++ /+	+		-	0/-?	-	food/0		+/0	0		+		17-38
Dermestidae	*Dermestes (2)*	f+m	f+m	faeces									-		-		+	light: - females: -	39
Nitidulidae	*Carpophilus (9)*	m	f+m	cells near trachea	+		0	+			-	fermenting food					+	starvation: +	40-53
Platypodidae	*Platypus (3)*	m	f+m	hindgut / anus						+		food							54-56
Scarabaeidae	*Cotinus (1), Maladera (1), Orytes (2), Popillia (1)*	f/m/ f+m	f+m			++			0?			fermenting food			0?		+		57-60
Scolytidae	*Dendroctonus, Gnathotrichus, Ips, Orthotomicus, Pityogenes, Pityokteines, Scolytus, Tomicus, Trypodendron (approx. 100)*	f/m/ f+m	f+m	fecal pellets	+	++ /+	+	-/0	-/0	-/+	-/+	food / resin / damaged wood	+/0	+/0		+/-	+	decay odour: - pheromone: - males present: -	61-70
Tenebrionidae	*Tribolium (6), Blaps (2)*	m/ f+m	f+m/ f+m+1	gland on prothorax	+		0/ +	0/+	-				+	0	0	-	+/ --	daytime: +	71-80

Taxa		Signal transmission			Factors affecting emission					Factors affecting response						Refs
order and family	genera (number of species per genus)	emitter	responder	origin	cross attraction	food	age	maturity	mating	females present	males present	co-attractant	age	maturity	mating	crowding	dose	other	
Collembola																			
Entomobryidae	*Orchesella (2)*	f+m	f+m		–							food / water	moulting					humidity: –	81–83
Hypogastruridae	*Hypogastrura (1)*	f+m	f+m															humidity: –	84–85
Onychiuridae	*Onychiurus (1)*	f+m	f+m									food / water	moulting					humidity: –	86
Tomoceridae	*Tomocerus (1)*	f+m	f+m		+														87–88
Dermaptera																			
Forficulidae	*Forficula (1)*		f+m+l	frass, m cuticula															89
Dictyoptera																			
Blattellidae	*Blattella (3), Periplaneta (1), Supella (1)*	f+m+l	f+m+l	faeces, supra-anal plate	+		0	gravid –					– / 0				+		90–93
Diptera																			
Anthomyiidae	*Delia (1)*	f	f (m:nt)	ovipositor								microbial rot		++	++				94–96
Calliphoridae	*Lucilia (1)*	f	f (m:nt)			+		+	+			carrion		++					97
Culicidae	*Aedes (4), Culex (3)*	e / l / f	f	e: apical droplet	+				++(f)			breeding substrate		++					98–100
Drosophilidae	*Drosophila (27)*	m+ mated f	f+m	m:ejaculatory bulb	+		+	0 / ++	++(f) + / 0 (m)	+	0	fermenting substrate	0	0			+	starvation: +	101–115
Glossinidae	*Glossina (1)*	l	f	anal exudate								moisture						only in dry season	116
Psychodidae	*Lutzomyia (1), Phlebotomus (1)*	f	f	accessory gland / maxillary palps			e: –					breeding substrate							117–119
Simuliidae	*Simulium (2)*	e / f?	f		+		–												120–123

Taxa		Signal transmission			Factors affecting emission							Factors affecting response						Refs
order and family	genera (number of species per genus)	emitter	respon- der	origin	cross attraction	food	age	matu- rity	mating	females present	males present	co- attractant	age	matu- rity	mating	crow- ding	dose	other	
Heteroptera																			
Coreidae	*Leptoglossus (1)*, *Serinetha (1)*	m / l	f+m+l / f+l																124-126
Lygaeidae	*Oncopeltus (1)*	l+f	l+f (m: nt)		-		-	-					-	-					127
Pentatomidae	*Bagrada (1)*, *Biprorulus (1)*, *Eurydema (1)*, *Euschistus (6)*, *Nezara (1)*, *Plautia (1)*, *Podisus (5)*, *Stiretrus (1)*	l / m / m / f+m / l+m	l / f+m+l / f+m / f+m / m+f+l	DAG *	+		+		0	0		tactile stimuli	l: - / moul- ting	0			--	light: -	128-139
Pyrrhocoridae	*Dysdercus (2)*	f+m+l	f+m+l	DAG *													--		140-141
Reduviidae	*Pristhesancus (1)*, *Rhodnius (1)*, *Triatoma (5)*	m / f+m+l	f+m / f+m+l	DAG * / faeces	+	+	0								- (for some days)			starvation: + light, shelter: +	142-146
Homoptera																			
Aphididae	*Aphis (1)*, *Brevicoryne (1)*, *Hyadaphis (1)*, *Lipaphis (1)*, *Phorodon (1)*, *Rhopalosiphus (1)*, *Sitobion (1)*	apt / ala / gym / spr.mig[a]	apt / ala / gym / spr.mig[a]	cornicles	-							plant: 0		0 (apt[a])		- (ala[a])			147-152
Hymenoptera																			
Aphidiidae	*Lysiphlebus (4)*	f	f		+													hosts: -, plant: 0, non-host: 0, light: +	153
Chalcididae	*Brachymeria (2)*	f+m	f+m						0									light: -	154-155
Ichneumonidae	*Megarhyssa (1)*, *Rhyssa (1)*	m	m		+					+	+	fungi							156-157
Orthoptera																			
Acrididae	*Hieroglyphus (1)*, *Locusta (1)*, *Schistocerca (1)*	f+m / l / m / f+m	f+m / l / f+m / f	faeces	+		l: 0 / m: +	m: +?		+	+		0	0 / +			+	phase: 0	158-166

Taxa		Signal transmission			Factors affecting emission					Factors affecting response					Refs
order and family	genera (number of species per genus)	emitter	respon- der	origin cross attraction	food	age	matu- rity	mating	females present	males present	co- attractant	age	matu- rity	mating	crow- ding	dose	other
Thysanoptera																	
Thripidae	*Thrips (2)*	m	m(+f?)				+				landmark, flowers		+				167

Abbreviations:

m=male, f=female, l=larva/nymph, e=eggs, nt=not tested, ++=pre-requisite, +=increase, 0=no effect, -=decrease, --=dispersive effect, ?=uncertainty

*= dorsal abdominal gland; °=aphid life stages: apt=apterous, ala=alatae, gym=gymnoparea, spr.mig=spring migrants

References:

1–6: Dendy et al. 1989, Cork et al. 1991, Dowdy et al. 1993, Mayhew and Phillips 1994, Boughton and Fadamiro 1996, Smith et al. 1996; **7:** Peng and Weiss 1992; **8:** Copp 1983; **9–16:** Pierce et al. 1983, Millar et al. 1985, Oehlschlager et al. 1988, Pierce et al. 1988, White and Chambers 1989, Chambers et al. 1990, Pierce et al. 1991a, b; **17–38:** Selander 1978, Phillips and Burkholder 1981, Faustini et al. 1982b, Booth et al. 1983, Phillips et al. 1984, Walgenbach and Burkholder 1986, Blight and Wadhams 1987, Dickens and Wiygul 1987, Phillips et al. 1987, Trung et al. 1988, Barnes and Capatos 1989, Dickens 1989, Dickens and Prestwich 1989, Roseland et al. 1990, Rochat et al. 1991a, Budenberg et al. 1993, Gries et al. 1993, Weissling et al. 1993, Eller et al. 1994, Oehlschlager et al. 1995, Eller and Bartelt 1996, Ramirez-Lucas et al. 1996; **39:** Rakowski and Cymborowski 1986; **40–53:** Bartelt et al. 1990a, b, Dowd and Bartelt 1991, Bartelt et al. 1992, Lin et al. 1992, Bartelt et al. 1993a, b, Blumberg et al. 1993, Dowd and Bartelt 1993, Bartelt and James 1994, Bartelt et al. 1994, Petroski et al. 1994, Bartelt et al. 1995, Williams et al. 1995; **54–56:** Renwick et al. 1977, Milligan et al. 1988, Milligan and Ytsma 1988; **57–60:** Iwabuchi and Takahashi 1983, Domek and Johnson 1988, Yarden and Shani 1994, Hallett et al. 1995; **61–70:** Nijholt 1970, Borden 1974, Elliott et al. 1975, Vite and Francke 1976, Wood 1982, Byers 1983, Raffa and Berryman 1983, Byers 1989b, Schlyter and Birgersson 1989, Kostyk et al. 1993; **71–80:** Kaufmann 1966, Ikan et al. 1970, O'Ceallachain and Ryan 1977, Faustini et al. 1981, Faustini et al. 1982a, Mondal and Port 1984, Suzuki et al. 1987, 1988, Trung et al. 1989, Obeng-Ofori and Coaker 1990b; **81–83:** Joosse 1970, Verhoef and Nagelkerke 1977, Verhoef et al. 1977; **84–85:** Joosse 1970, Mertens and Bourgoignie 1977; **86:** Joosse and Koelman 1979; **87–88:** Verhoef and Nagelkerke 1977, Verhoef et al. 1977; **89:** Walker et al. 1993; **90–93:** Rust and Appel 1985, Ross and Tignor 1986, Sakuma and Fukami 1991, 1993; **94–96:** Hough et al. 1981, Hausmann and Miller 1989b, Judd and Borden 1992; **97:** Barton Browne et al. 1969; **98–100:** Bentley and Day 1989, Pile et al. 1991, Klowden 1999; **101–115:** Bartelt and Jackson 1984, Bartelt et al. 1985a, b, 1986, Moats et al. 1987, Schaner et al. 1987, Bartelt et al. 1988, 1989, Schaner et al. 1989a, b, c, d, Jaenike et al. 1992, Schaner and Jackson 1992, Hedlund et al. 1996b; **116:** Leonard and Saini 1993; **117–119:** Schlein et al. 1984, Elnaiem and Ward 1991, Dougherty et al. 1993; **120–123:** Coupland 1992, McCall et al. 1994a, b, McCall 1995; **124–126:** Dhawan and Gandhi 1989, Yasuda 1990, Yasuda and Tsurumachi 1995; **127:** Aller and Caldwell 1979; **128–139:** Ishiwatari 1976, Evans and Root 1980, Harris and Todd 1980, Aldrich et al. 1984, Moriya and Shiga 1984, Lockwood and Story 1985, Dhiman and Gandhi 1988, Kochansky et al. 1989, Aldrich et al. 1991, James et al. 1994b.; Ishiwatari, 1974, Aldrich et al. 1995; **140–141:** Bala and Gandhi 1988, Farine et al. 1992; **142–146:** Schofield and Patterson 1977, Cruz Lopez et al. 1993, James et al. 1994a, Lorenzo Figueiras et al. 1994, Lorenzo and Lazzari 1996; **147–152:** Kay 1976, Pettersson and Stephansson 1991, Campbell et al. 1993, Pettersson 1993, Pettersson et al. 1995, Hardie et al. 1996; **153:** Stary and Volkl 1988; **154–155:** Simser and Coppel 1980, Mohamed and Coppel 1987a; **156–157:** Madden 1968, Davies and Madden 1985; **158–166:** Fuzeau-Braesch et al. 1988, Nayak and Gandhi 1990, Byers 1991, Obeng-Ofori et al. 1993, Obeng-Ofori et al. 1994a, b, Torto et al. 1994, Deng et al. 1996, Njagi et al. 1996; **167:** Kirk 1985.

Some species produce volatiles that mimic a co-attractant, e.g., fungal volatiles (Cucujidae: Pierce et al. 1989). For species whose aggregations are not food-based, adults have a fully gregarious lifestyle (Scarabidae: Hallett et al. 1995) or the aggregations are formed in sheltered places during winter (Coccinellidae) or daytime (Tenebrionidae). The aggregation pheromones in these tenebrionid species are defensive secretions when released in high concentrations, and additionally act as alarm pheromones, causing immediate dispersal in conspecifics (Kaufmann 1966, Ikan et al. 1970). In a dermestid species, the aggregation pheromone also acts as a primer pheromone, influencing growth rate and development of the larvae (Rakowski and Cymborowski 1982).

Collembola

Collembola are soil-living wingless insects. Water saturation levels of the soil affect survival, respiration and reproduction. The reported species spend periods of their life gregariously, as during moulting (Entomobryidae, Hypogastrura: Joosse 1970, Verhoef and Nagelkerke 1977, Verhoef et al. 1977). In or directly after these periods, they reproduce. In all mentioned species, both males and females produce and respond to the pheromones.

Dermaptera

Males, females and nymphs aggregate in dark and damp places, using pheromone. The earwigs possess defensive secretions that may also act as alarm pheromones (Walker et al. 1993).

Dictyoptera

The aggregation pheromones of cockroaches comprise attractant and arrestant components (Sakuma and Fukami 1991). Adults of both sexes and nymphs all produce and respond to the pheromone in a similar manner (Sakuma and Fukami 1993). Not all cockroaches use aggregation pheromones (Rust and Appel 1985).

Diptera

In most dipteran species, aggregations are primarily formed by ovipositing females. The most notable exception is a psychodid species, where females release a volatile aggregation pheromone from the maxillary palps during blood-feeding, that attracts other females but not males (Schlein et al. 1984). The aggregations of ovipositing females arise from pheromones of adult females (Anthomyiidae, Calliphoridae, Drosophilidae, Psychodidae), or from pheromones of eggs, larvae or pupae (Culicidae, Glossinidae, Simuliidae). Breeding substrates are often co-attractants. In Culicidae and Drosophilidae, the pheromones of the females are in fact male-derived: During copulation, males transfer the pheromone to the females. The pheromone load transferred to drosophilid females is usually large (30-70% of the total storage in males), and females emit the majority of pheromone within hours, attracting both males and females. Male drosophilids also emit small amounts of the pheromone and these are sufficient to attract conspecifics. In three species, however, only minor amounts are transferred to females, and males are the main emitters. In these species emission is increased by the presence of females (Schaner and Jackson 1992). In several drosophilid species virgin females produce other volatiles that are also slightly attractive to both sexes, but

considerably less so than the male-produced pheromones (Bartelt et al. 1985b, 1986, Moats et al. 1987, Schaner et al. 1987, Bartelt et al. 1988, 1989, Schaner et al. 1989c, Hedlund et al. 1996b), except for one species where females are also very attractive (Bartelt et al. 1989). In nature, drosophilid aggregations of both males and females develop on a substrate, on which they feed, mate and oviposit (Spieth 1974).

Apart from the pheromone deposition, females of two families inoculate the larval substrate with microbia, enhancing larval development (Anthomyiidae, Drosophilidae). Anthomyiid females preferentially oviposit on onions with low to moderate larval feeding damage and microbial growth, not on healthy or severely damaged plants, and this preference corresponds with the survival probability of the offspring (Hausmann and Miller 1989a). The glossinid females larviposit fully mature larvae, just before pupariation (Leonard and Saini 1993). Ovipositing in calliphorid groups is at least partly sustained by contact between the females via contact pheromones (Barton Browne et al. 1969).

Heteroptera

In Heteroptera, often males, females and nymphs respond to the aggregation pheromones. The production is either by both sexes and all developmental stages or restricted to males or nymphs. Aggregations sometimes break up before reaching maturity (Lygaeidae: Aller and Caldwell 1979) or are associated with the moulting of nymphs (Pentatomidae: Evans and Root 1980). Pheromones often originate from specialized glands, that are also associated with defensive secretions and alarm pheromones.

The biology of the aggregation pheromone-possessing heteropterans is diverse. Some species are herbivorous (Coreidae, Lygaeidae, Pentatomidae), others are predatory (Pentatomidae, Reduviidae) or haematophagous (Reduviidae), some exhibit communal feeding and some are solitary feeders. Most aggregations are formed on food sources. In Reduvidae, however, aggregative behaviour occurs in daytime shelters (Lorenzo Figueiras et al. 1994). Defecation typically occurs just outside the shelters, and these shelters are more attractive than clean ones (Lorenzo and Lazzari 1996).

Some species posses pheromones that are aggregative at low concentrations, but dispersive (alarm) (Pentatomidae: Ishiwatari 1974, 1976, Lockwood and Story 1985, Aldrich et al. 1995) or defensive (Coreidae: Miller 1956, Aldrich 1988) at high concentrations. Species of Heteroptera show mostly an immediate dispersive response to a (sudden) increase in the dose of the aggregation pheromone, whereas most other insects have a positive dose-aggregative response curve within certain boundaries.

Homoptera

Aphids often live in large colonies of apterous (wingless) parthenogenic viviparous females, with a high degree of relatedness within the colony. Pheromones arrest other apterous females in the vicinity of a colony (Kay 1976) or deter them (Pettersson et al. 1995). The apterous females periodically produce winged offspring (alatae) that disperse to new plants. In two species an aggregation pheromone is emitted by alatae that attracts other conspecific alatae but not apterae or heterospecifics (Pettersson and Stephansson 1991, Pettersson et al. 1995). At the end of the season, host alternating species produce sexual migrants, gymnoparae. These form aggregations on the winter host by using pheromones (Pettersson 1993, Hardie et al. 1996). During spring migration, aggregation pheromones are involved in forming new colonies on food plants (Campbell et al. 1993).

Efficient resource use / overcoming host resistance

Helping in finding food has frequently been suggested as a function of the use of aggregation pheromones, for example in species of Cucujidae (Millar et al. 1985), Curculionidae (Selander 1978, Walgenbach and Burkholder 1986, Barnes and Capatos 1989, Dickens 1989, Roseland et al. 1990, Rochat et al. 1991b, Budenberg et al. 1993, Giblin Davis et al. 1994), Dermestidae (Rakowski 1988), Tenebrionidae (Obeng-Ofori and Coaker 1990b) and Psychodidae (Schlein et al. 1984). Formulated as such, this implies altruistic behaviour and group selection and should therefore be avoided (see above). Besides, the deduction is not consistent with the observation that responses are often only found when the pheromone is accompanied by food odours: If they only respond when they can perceive the food odours, why would they need pheromones, and how can it explain the preference for food odours with pheromones above food odours alone?

Nonetheless, the use of aggregation pheromones indeed results in communal feeding in many insect species. Frequently, food is required for the production of pheromone and food odours are required for responses. Therefore, it is probable that under certain circumstances, resource exploitation can promote aggregative behaviour. These circumstances should be such that individual efficiency is enhanced in aggregations. Reduced search time for food is one way to achieve increased efficiency, and it has indeed been proposed as an important benefit of group living in patchy, unpredictable environments, especially if costs of competition are low, for example because patches are large relative to individual requirements, or ephemeral relative to the time it takes to exhaust them (Pulliam and Caraco 1984). For the insects in our survey, many other explanations seem even more applicable.

A benefit of communal feeding can comprise the reduced *per capita* cost of initiating attack on a new food source. For example, chrysomelid species feed on leaves that are covered with a wax layer and tend to feed all on the same cotyledon (Anderson et al. 1992). Possibly gregarious feeding can be more successful in opening the wax layer (Peng and Weiss 1992). Similarly, nymphal survival in lygaeid bugs is increased in larger groups when their food source, seeds, is offered as unopened pods. Only by the joint secretion of lytic enzymes onto unopened pods, the nutrients of the seeds become available for ingestion (Ralph 1976). In aphids, saliva injection is reduced on previously infested leaves (Prado and Tjallingii 1997). However, this reduced cost of initiating attack yielded no clear benefits in a field study (Hodgson and Godfray 1999).

Communal feeding can also yield profits with respect to the host defence machinery. The benefits of aggregation pheromones in barkbeetles (Scolytidae) are the best documented ones and commonly known as 'overcoming host resistence' (Borden 1974, Byers 1989a). The aggregated attack by beetles surmounts host tree defence mechanisms (resin flow, toxins) through the joint inoculation of the tree with deadly fungi, which the pheromone-producing sex carries in highly specialized mycangia (Wood 1982). In healthy trees, single beetles have no chance to succeed colonization and reproduction, but a rapid communal attack kills the tree and renders it suitable for feeding of both the adults and the larvae (Borden 1974, Raffa and Berryman 1983, Byers 1989a). A remarkable feature that should be noted is that related species attack dead trees and also use aggregation pheromones (Wood 1982, Landolt 1997). It is actually thought that aggregation pheromones must have occurred in an ancestral non-killing species, because the rapid mass-attack that is needed to kill a tree could impossibly have evolved without a pre-existing highly

efficient communication system (Schlyter and Birgersson 1989). Possibly this ancestral trait simply reflected a mate finding system (Wood 1982). Alternatively, it could be related to the linkage with carrying fungi in the mycangia. The frequent relationship between fungi and wood-feeding insects is thought to be reciprocally mutualistic: Fungi convert the often indigestible material into more readily available nutrients and the insects act as vectors for dispersal of the micro-organisms (Madden 1968, Kok 1979). Whether and why aggregation pheromones should be involved in this latter relationship is as yet unresolved (but see also 'similarities among taxa').

Additionally, aggregation can prevent food deterioration. For locusts (Orthoptera), it is suggested that, since plant chemical defence mechanisms can be induced by feeding, at increasing population densities, it might be beneficial to synchronize plant attack, and start feeding as a flock. A 'hit and run' strategy leaves plants no time to initiate defences (Rhoades 1985). Also, when aphids are clustered on the same leaves within a food plant, the plant survives throughout colony development, whereas dispersion of the same number of aphids across leaves causes earlier plant wilting and mortality, which leads to increased (risky) migration and lower individual and cumulative body weights (Way 1973). The more stable interaction with the host plant can probably be attributed to the sustenance of photosynthesis in the undamaged plant parts (Way 1973).

Finally, individuals can experience increased individual intake rates in groups. This is shown for several species op Heteroptera (Aldrich 1988). A more efficient atmospheric water intake in groups is reported for first instar nymphs of a pentatomid species, shortening their developmental time (Lockwood and Story 1986). In Homoptera, group feeding increases the nutritional status of the plant at the specific site of attack (Way 1973). Consequently, individuals grow larger when feeding in groups and intermediate sized colonies grow initially fastest, although later on crowding reduces reproduction (Way 1973). Increased intake rates are also suggested for communal blood-feeding in Diptera (Simuliidae: McCall and Lemoh 1997). For the haematophagous heteropterans, it was suggested that the aggregations in daytime shelters might be adaptive to mutually acquire the symbionts that are essential for normal development (Lorenzo and Lazzari 1998).

Mate finding

In some species, the onset of pheromone production or response is simultaneous with reaching sexual maturity (Cucujidae, Drosophilidae, Pentatomidae), or the presence of females and/or mating reduces the production (Curculionidae, Scolytidae, Tenebrionidae). Mating sometimes reduces the response to the pheromones (Dermestidae, Reduviidae). If only one sex produces the pheromone, it sometimes primarily attracts the other sex (Dictyoptera: Chow et al. 1976, Cucujidae: White and Chambers 1989). All this hints that the pheromone mediates the sexual interaction, especially mate finding (Faustini et al. 1981, Phillips and Burkholder 1981, Faustini et al. 1982b, Booth et al. 1983, Boake and Wade 1984, Rakowski and Cymborowski 1986, Blight and Wadhams 1987, Domek and Johnson 1988, Milligan and Ytsma 1988, Obeng-Ofori and Coaker 1990a, Roseland et al. 1990, James et al. 1994a, James et al. 1994b, Yarden and Shani 1994). If mate finding would be the main purpose of the pheromones, the attraction of males to male-produced pheromones can be seen as a by-product or cost of attracting females. Although

attraction of other males is not serving the emitter, it may evolve if the costs of 'not calling' are larger than the costs of 'having to share'.

Responses of the same sex as the emitter might have a function in mate finding as well. For a homopteran species (Pettersson 1993), pheromones of sexual females are attractive to both males and females. The response of females is suggested to be an attempt to increase efficacy by joining into communal calling for males, increasing the active space of the pheromones (see also Baker 1985), and the response of males to have a purely sexual function. The mating aggregations of Thripidae also seem to primarily serve female attraction.

The ichneumonid parasitoid males that gather around emergence site arise from mate searching (Crankshaw and Matthews 1981). The males use defensive mandibular secretions in interactions with con- and heterospecific males around the emergence sites, and these secretions attract yet other males of several species. All males patrol at the emergence sites and only after the females become exposed to the males, species recognition is achieved, again by the mandibular secretions (Davies and Madden 1985). This can be an example of secretions with an initial function (defensive), evolving towards an aggregation pheromone through exploitation by competitors.

For Orthoptera it has been hypothesized that the migration to new environments will favour increased genetic recombination. This could possibly explain the increased chiasma frequencies in the spermatocytes of gregarious phase individuals (Byers 1991, and references herein). Possibly aggregation gives females an additional benefit by providing the opportunity to choose from or multiply mate with a large number of males.

In various cases, being in a group simply facilitates mate finding, so that the sexual function is a secondary function, but therefore not unimportant in terms of saving energy (Harris and Todd 1980, Lorenzo and Lazzari 1996). In a pentatomid species, the mating system has been described as a resource based lek or polygyny (Aldrich 1988), and it is suggested that such a system with scramble competition for mates may have evolved because of the digestive advantage of group feeding (Aldrich 1988). Thus, what started of as a group-feeding-function subsequently acquired also a sexual function. Similarly, being in a winter aggregate allows coccinellids to mate early in the season, after which the highly dispersive adults can start searching for suitable oviposition sites (Hodek et al. 1996). The spermatheca in female insects allow them to store sperm after mating and utilize it over extensive periods of time. Collembola aggregate during moulting, and an additional sexual advantage to the use of aggregation pheromones is quite likely. Males deposit and leave their spermatophores for females to find and pick up. The chance of donating or finding is highly increased if the two sexes are close together instead of randomly distributed (Joosse 1970, Verhoef and Nagelkerke 1977).

In Dermaptera, Dictyoptera, Heteroptera and Orthoptera, aggregations comprise different developmental stages, making a purely sexual function unlikely. Field observations on drosophilid fruit flies in aggregations revealed frequent sexual interactions and copulations (Spieth 1974), but since sexually immature adults also produce and respond to the aggregation pheromone, a purely sexual function is improbable (Bartelt and Jackson 1984).

In still other species, it is unlikely that the pheromones play any role at all in sexual interactions, because no sexual interactions are observed after group formation (most dipteran families, Coreidae, Pentatomidae: Moriya and Shiga 1984), mated individuals show as strong a response as virgins (Bostrichidae, Curculionidae, Tenebrionidae), or nymphal aggregations break up before reaching maturity (Lygaeidae, Pentatomidae). The presence of separate volatile sex pheromones in approximately 10 % of the species (Tenebrionidae: O'Ceallachain and Ryan 1977, Selander 1978, Dermesitidae: Abdel-Kadar and Barak 1979, Hedin et al. 1979, Chalcididae: Mohamed and Coppel 1987b, Curculionidae: Trung et al. 1988, Coreidae: Dhawan and Gandhi 1989, Trung et al. 1989, Aphididae: Dawson et al. 1990, Pickett et al. 1992, Pentatomidae: Borges and Aldrich 1994, Acrididae: Inayatullah et al. 1994) could make a prominent sexual role for aggregation pheromones redundant. When considerable cross attraction occurs (i.e. low specificity), a primary sexual function for the pheromone is perhaps not to be expected.

In Culicidae and Drosophilidae, a sexual interaction between males and females is reported, that is exactly the opposite of mate finding. Males transfer anti-aphrodistic pheromones to the females during copulation and decrease their receptivity to other males ('chemical mate guarding'), although in Drosophilidae the same pheromone also brings the two sexes together. In both taxa, the transferred pheromones of the males are also involved in the aggregated ovipositing in females (Zawistowski and Richmond 1986, Bentley and Day 1989, Ferveur et al. 1989), but see also (Scott and Richmond 1987).

Protection from natural enemies

Being in a group can be advantageous with respect to risk of predation or parasitism in several different ways. Firstly, when individuals can engage in active defence, the chances of successfully counter-attacking natural enemies might be larger in larger groups. Furthermore, warning or aposematic colouration to fend off an attack may be a more effective strategy if exploited in a group, either because a group allows for quicker/easier learning by the predator (larger stimulus input), or because the chance of being encountered by an *experienced* predator is larger. Alternatively, sufficiently large groups of insect that are not capable of active defence are sometimes less efficiently exploited by predators or parasitoids (dilution of risk), i.e. the selfish herd (Hamilton 1971). Additionally, being in the centre of a group can result in a lower chance of being captured than at the margins. Finally, by being surrounded by others, the vigilance of the group as a whole can increase, enhancing the chance of spotting and fleeing from a natural enemy before it initiates attack.

As sound as these ecological strategies might seem, the evolution of several of these mechanisms might be hampered by severe costs at intermediate group sizes, only reaching net benefits at much higher densities (Sillén-Tullberg and Leimar 1988). In most cases, being in a group also increases the conspicuousness to natural enemies, most notably with aposematic colouration, and this could easily create an unbridgeable hurdle in the evolution of grouping. In addition, some of the strategies seem to be founded on group selection theory and would not be evolutionary stable and liable to cheaters (e.g., vigilance, risk in the centre versus margins, risky active defences). Relatedness between the individuals might be required before such a strategy could evolve (kin selection). Even though the evidence of an eventually decreased attack by natural enemies can be convincing, the evolution of aggregative behaviour in terms of avoiding predation is not always explicable, and might in fact originate from alternative or additional benefits.

Afterwards, selection resulting from predation could further enlarge optimal group sizes (Sillén-Tullberg and Leimar 1988).

Active defence and unpalatability in insects is executed mainly by means of chemicals. From the species in our survey, most heteropteran species (Aldrich 1988), the earwigs (Dermaptera) (Walker et al. 1993), coccinellids (Hodek et al. 1996) and two tenebrionid beetle species (Kaufmann 1966, Ikan et al. 1970, Keville and Kannowski 1975) possess defensive secretions, that are either pungent, irritating or burning. The marked colouration of their elytra is aposematic. In Heteroptera these secretions seem to be directed mainly to arthropod predators, especially ants (Ishiwatari 1974, Aldrich 1988, Farine et al. 1992), but against certain natural enemies, chemical defence only marginally reduced the rate of attack. In a pentatomid bug species, the *per capita* risk of predation for some but not all predators was significantly lower in aggregated nymphs than in solitary nymphs, because of active defence (Lockwood and Story 1986). For another heteropteran species, however, the aggregating nymphs could benefit at best (if at all) from dilution of risk (Ralph 1976). Rejection of Heteroptera and Coccinellidae by vertebrate natural enemies (e.g., birds and shrews) is primarily based on distastefulness (Aldrich 1988, Hodek et al. 1996).

In Orthoptera, the bright colouration of the gregarious phase could provide a non-specific Batesian mimicry, as the large swarm of aposematically coloured, buzzing insects, might scare off predators (Byers 1991). Alternatively, local predators might soon be satiated, decreasing the *per capita* risk of predation for gregarious locusts (Byers 1991). A related species, also exhibiting solitary and gregarious phases, feeds preferentially on a host plant species that renders them unpalatable to lizards, and lizards quickly learn to avoid the unpalatable aposematic colour morph (Sword 1999). The phenotypic plasticity that is found in the colour morphs of locusts, cryptic at low densities and aposematic at high densities, might have facilitated the evolution of grouping and aposematism, balancing the costs of increased conspicuousness and the benefits of predator avoidance for different densities (Sword 1999).

In many coleopteran and dipteran species, cockroaches (Dictyoptera) and for overwintering aggregations in general, dilution of risk has been loosely suggested as a benefit of group living. Again, evolution of such a strategy is not easily explained, because at (initial) low prey density, benefits are essentially absent, while costs could already arise. Furthermore, dilution of risk is only conceivable as an effective anti-predator strategy when the aggregation of natural enemies does not match the aggregation of their prey. A field study on an aggregating aphid species without alarm pheromones did show a significant and considerable increase in population growth rates with increasing colony size, and by careful experimentation this could be attributed to dilution of risk, even though the predator did show an increase in numbers and feeding rate at higher prey densities (Turchin and Kareiva 1989). A similar study on another aphid species, possessing aggregation pheromones, did not show a dilution effect, but colony sizes and predator densities were lower (Hodgson and Godfray 1999). The high relatedness within aphid colonies could facilitate evolution of gregariousness.

In both Homoptera, Heteroptera and the tenebrionids, the insects often simultaneously possess aggregation and alarm pheromones, sometimes being the same compounds but in different concentrations (Ishiwatari 1974, 1976). The alarm pheromones in these insects cause mostly an immediate dispersive

behaviour, in both conspecifics and heterospecifics (Ishiwatari 1974) suggesting that they might benefit from group living by increased vigilance.

Protection from environmental conditions

Insects can alter their physical micro-environment to a certain degree, in particular they can raise the ambient temperature and relative humidity in a confined space. Insects evaporate water through their spiracula, making them desiccation prone. Desiccation proceeds more slowly at high humidity. In groups, all insects evaporate to some extent, and the rise in relative humidity can reduce the desiccation risk for each group member. Since insects are homothermic, an increase in ambient temperature increases activity and metabolic activity.

Several stored product pest species (Coleoptera) can create so called 'hot spots', because by forming a group, ambient relative humidity and temperature is considerably increased locally (Howe 1962). These conditions might be more benign or induce faster metabolism and larval development (Howe 1962, Sinha and Wallace 1966, Plarre 1996). Alternatively, indirect benefits could arise, namely improved conditions for growth of fungi and micro-organisms (Sinha and Wallace 1966) on which they form aggregations and feed (Surtees 1964) or modified accessibility of food through lower seed coat resistance (Sinha and Voisey 1978). The indirect cases would fall in the category of improving efficient resource use.

In one heteropteran species, larvae developed faster in aggregations at low temperature, indicating that aggregations provided warmer conditions (Lockwood and Story 1986). In that same species, desiccation tolerance in stressed conditions was higher in grouped individuals than in solitary ones (Lockwood and Story 1986). In Collembola, a striking congruence was found between species for the degree of dessication susceptibility and the degree of aggregation (Joosse 1970). Additionally, aggregation was strongest within species during moulting when they are especially prone to dessication (Joosse 1970).

For the aggregations in shelters (Tenebrionidae, Dictyoptera, Dermaptera, some heteropteran and hymenopteran species), decreasing the risk of desiccation could be proposed as a benefit of forming aggregations. The same was proposed for Coccinellidae, but measurements of temperature and relative humidity inside the group were not different from outside the group (Copp 1983).

Benefits associated with aggregated oviposition

Aggregated ovipositing results in three levels of aggregation: The ovipositing females form aggregations, the eggs are subsequently clustered and the hatched larvae remain often aggregated until metamorphosis. The benefits of aggregated ovipositing do in fact coincide with all previous categories, but to different degrees for each level of aggregation and each species.

EFFICIENT RESOURCE USE: For both Drosophilidae (Sang 1956) and Anthomyiidae (Hausmann and Miller 1989a, Judd and Borden 1992), larval food quality is improved by the presence of other larvae. The larvae of Drosophilidae might cultivate their resource by reducing fungal contamination through ploughing and tunnelling through the substrate, and are more efficient in doing so in a group (Ashburner 1989, B. Wertheim, personal observation). Intermediate larval densities resulted in the lowest mortality and shortest developmental period (Sang 1956). The adults of Drosophilidae are reported to change the

breeding resource to the advantage of larvae (Schaner and Jackson 1992) and females of both taxa transmit and inoculate micro-organisms in larval hosts (Gilbert 1980, Hough et al. 1982). Also for sandflies (Psychodidae), high larval densities reduced fungal contamination (Elnaiem and Ward 1991). Mosquito larvae in groups prevent the formation of scum on their euthropic food sources (McCall and Cameron 1995).

MATE FINDING: Synchronization of hatching has been proposed to benefit mate finding for Simuliidae (McCall et al. 1994b, McCall 1995) and Orthoptera (Byers 1991). Such a hypothesis cannot apply for species, where maturity is only reached after several days or at different ages for the two sexes (Drosophilidae: Bartelt and Jackson 1984, Moats et al. 1987, Bartelt et al. 1989).
Female drosophilids in groups seem less harassed by courting males and may achieve higher oviposition rates (B. Wertheim, personal observation, chapter 3).

PROTECTION FROM NATURAL ENEMIES: Females of Simuliidae are especially prone to predation by fish and insects during ovipositing (Davies 1962, Golini and Davies 1987), and large group sizes might dilute the *per capita* risk (McCall and Cameron 1995). Also for sandfly females and eggs, dilution of risk is suggested as a function of the aggregations (Elnaiem and Ward 1991). The eggs in egg masses of Simuliidae are attacked from the outside by predators, yielding the advantage to eggs in the centre (McCall et al. 1994b, McCall 1995). At mass emergence, individuals in a swarm could again experience diluted risk of predation (Orthoptera: Byers 1991, Diptera: McCall and Cameron 1995) .

PROTECTION FROM ENVIRONMENTAL CONDITIONS: The eggs of Simuliidae are very susceptible to desiccation and cannot withstand prolonged exposure to air (Imhof and Smith 1979, Golini and Davies 1987). Most species oviposit in or just above a water source (Davies 1962, Golini and Davies 1987, Baba and Hiroyuki 1991). The eggs that are deposited in communal egg masses can benefit from the properties of an egg mass, i.e. gelatinous outer layer and smaller ratio for volume : surface area, which are both thought to reduce risk of dessication (McCall et al. 1994b, McCall 1995). Additionally, on floating debris or plants trailing in streams, the weight of the mass slightly lowers the egg mass into the water (Davies 1962, Golini and Davies 1987 , McCall et al. 1994b) while the stem and egg masses together just float and thus ensure warming by the sun (Davies 1962). Finally, the eggs in the centre of the mass will be protected from desiccation by outer layers of eggs when water levels fall (McCall and Cameron 1995).

The aggregated larvipositing of tsetse flies is somewhat different from the other Diptera, since the larvae almost immediately pupariate after larviposition. Resource exploitation and also competition for food do not play a role, since the larvae develop fully and singly in utero. The aggregated distribution is only observed in the dry season, and responses to pheromones were only recorded on wet sand (Leonard and Saini 1993). Because of viviparity, females have an extremely low reproductive output (at most 20 offspring), and survival of their progeny is likely to be a major selective pressure for these insects. What mechanisms might provide higher survival probabilities of puparia in groups during the dry season is unclear.

Similarities within taxa

The similarities in mechanisms and benefits between species of the same family and even between families of the same order are immediately apparent. In Coleoptera, the response is highly food dependent; in Collembola, the relation to desiccation tolerance is striking; in Diptera, the use of aggregation pheromones often results in aggregated ovipositing; in Heteroptera, the aggregation pheromones are often defensive secretions in high concentrations; and in Heteroptera and Homoptera, sometimes grouped together as Hemiptera, aggregation pheromones are often accompanied by alarm pheromones. Hence, there is a clear phylogenetic predisposition.

Similarities among taxa

The species that were reported to use aggregation pheromones belonged to various trophic groups: Herbivores feeding on healthy plants and fruit, healthy and dead trees, damaged plants, trees or fruits, and stored products (e.g., grain, dried cassava, peanuts); predators and haematophagous insects; fungivores and detrivores, feeding on micro-organisms and mycelium. Within each trophic group, species of different taxa show remarkable similarities, indicating convergent evolution.

The number of species with aggregation pheromones that are somehow associated with microbial or fungal growth is quite striking. In about a quarter of the investigated families and over half of the investigated species, feeding is either directly on microbes or fungi in plants, trees or fruit, or infection is associated with feeding by the insect. For the anthomiid species, microbial decomposition enhances larval survival and development rates. The females inoculate substrates and 'coat' their eggs with micro-organisms and preferentially oviposit on already infested host plants or in groups of females (Hausmann and Miller 1989a, Judd and Borden 1992). The microbes are thought to predispose healthy onions to more successful attack by larvae, since healthy bulbs cannot be penetrated by larvae (Finch and Eckenrode 1985), or make available certain nutrients that enhance larval survival. A very similar story holds for the drosophilid fruit flies, where the inoculation of a substrate with yeasts improves the substrate for larval resource exploitation (Begon 1986), and adults are known to do so. Simultaneous ovipositing could enhance yeast infestation beyond a level achieved by a single female and that could enhance larval survival and growth.

The stored product coleopteran species change their micro environment by aggregating, increasing humidity and temperature. Although the modified micro-environment has mostly been linked with a increase in metabolic processes and development, it could also be seen as a mechanism to improve conditions for mould development or food accessibility. The occurrence of aggregation pheromones in stored product species coincides to a very large degree with the occurrence of adult feeding and reversibly, when adults do not feed, they possess sex pheromones rather than aggregation pheromones (Levinson and Levinson 1995). Bark beetles also inoculate the trees that they attack with fungi, upon which both adults and larvae thereafter feed; these fungi can be essential to recycle nutrients from the trees and can also be involved in killing living trees (Kok 1979, Wood 1982). The beetles have highly specialized structures to carry fungal inocula (Wood 1982). Nitidulid beetles feed preferentially on fermenting substrates (e.g., overripe fruit, damaged corns), and several microbial, in particular yeast volatiles are highly attractive (Dowd and Bartelt 1991, Nout and Bartelt 1998). They also are reported to transmit fruit-degrading

yeasts onto the substrates themselves (Michailides et al. 1992, Bartelt et al. 1993b). After colonization of a fruit, the beetles reproduce and larvae appear (Bartelt et al. 1994). Maybe the aggregative behaviour enhances inoculation success, and renders the fruit more suitable for larval development, as is hypothesized for Drosophilidae. The same could apply for one of the scarabeid beetle species, where the adults attack coconut and oil palms, form aggregates and can eventually kill the tree. They breed in decaying and fermenting matter, such as rotting palms (Hallett et al. 1995).

Irrespective of trophic group, about a third of the aggregative insects were vectors for pathogens and parasites of the host they fed on. For one species it was shown that the aggregative behaviour was not induced or affected by the presence or absence of the parasite, suggesting aggregation is not a behaviour invoked by the pathogen or parasite. It remains to be determined whether parasites and pathogens are transmitted disproportionally often by aggregative insects. If so, this indicates that the pathogens could benefit from aggregative behaviour of their vector insect. In some bacterial and fungal rots, a threshold for establishment has been reported, consistent with a benefit for pathogens of aggregative vectors (Atlas and Bartha 1993, Fleischer et al. 1999).

For insects feeding on healthy plants and leaves, it is relatively exceptional to use aggregation pheromones, considering the large number of insect species in this niche. For the species that possess these pheromones, aggregation behaviour is often linked with resource exploitation, either by increasing food quality (aphids), increasing efficacy (lygaeid bugs and chrysomelid beetles) or preventing food deteriorating (grass hoppers and aphids). This low incidence of leaf-feeding among insects with aggregation pheromones is in contrast to the frequent aggregations of caterpillars that arise from clutch laying butterflies (Stamp 1980). One might expect aggregation pheromones to occur more often in plant feeders, since rapid population build up might impair the inducible defensive ability of the plant (Rhoades 1985).

Carnivory (predation and parasitism) and haemotophagy is found in several families in our survey (Coleoptera, Diptera, Heteroptera, Hymenoptera), but aggregation is often during the inactive phase (seasonally, daily or during moulting) (Schofield and Patterson 1977, Evans and Root 1980, Lorenzo Figueiras et al. 1994, Hodek et al. 1996, Lorenzo and Lazzari 1996) or in a non-predatory developmental stage (parasitoid adults, dipteran larvae). Aggregated blood feeding is mediated in one species, but it remains unclear whether that is related to improved efficiency (or reduced host resistence/defence) or to reduced risk on mortality. In general, it seems that predation by non-social insects does not often give rise to aggregated food exploitation.

Active defence or unpalatability was found in a number of aggregative insects, among several orders and families. Both were frequently accompanied by aposematic colouration. Defensive secretions that act as aggregation pheromones when emitted at low concentrations were found in several heteropteran and one coleopterna family (Tenebrionidae). The colonies of Homoptera and the clutch laying in many Heteroptera results in higher relatedness between members of the aggregation, which could facilitate the evolution of aposematic colouration, unpalatability and vigilance through kin selection.

Cross attraction between taxa within a family, frequently by sharing components in the aggregation pheromones, was found in 9 families and 5 orders (Cucujidae, Nitulidae, Scolytidae, Tenebrionidae, Tomoceridae, Blattellidae, Drosophilidae, Reduviidae, Chalcididae). Possibly cross attraction reflects a constraint: The overlap in pheromone compounds between related taxa hampers the responders to properly distinguish between them. More plausible, however, is the hypothesis that cross attraction is also adaptive, i.e., the individual also gains some advantage from group forming with heterospecifics, since most proposed functions do not hinge on species specificity. This was suggested for the egg masses of blackflies and other dipterans (McCall 1995, McCall and Cameron 1995).

Costs of the use of aggregation pheromones

Each trait in nature is shaped by a balance between benefits and costs. Cost can arise as a direct consequence of the trait or because of a trade off. Additionally, costs can be physiological and ecological. In this section we will mainly focus on direct costs in an ecological perspective. The phenomenon of reduced pheromone production per individual under crowded conditions in many Coleoptera (e.g. Pierce et al. 1989, Mayhew and Phillips 1994, Bartelt et al. 1995), the repellent effect of high concentrations of pheromones (Cucujidae) and the mentioning of anti-aggregation pheromones (Scolytidae) indicate that after reaching a certain group size, the net gain of aggregation diminishes.

Competition for food, space, mates

Within aggregations, individuals frequently experience more severe competition than they would if they were solitary. The competition can be for food, space and mates. The larvae of Scolytidae (Wood 1982, Raffa and Berryman 1983, Byers 1989a, b) and Drosophilidae (Atkinson 1979, Grimaldi and Jaenike 1984) frequently experience severe intraspecific competition for food, and can even exhaust their food source before larval development is complete. Population dynamics of aphids (Homoptera) are thought to be mainly regulated by intraspecific food competition, through the effect on adults size and fecundity (Dixon et al. 1996). In Heteroptera, competition for space has been reported. Individuals fell off plants more easily when aggregated in multilayer clusters during strong winds (Lockwood and Story 1986). The females of blackflies (Simulidae) form huge aggregations while egg laying, and strongly compete for space (Muirhead-Thomson 1956), during which they can get entangled in the egg masses and die (Davies 1962). The mating system of Thysanoptera is thought to be a polygyny, with a limited number of males getting all the matings.

Transmission of diseases/parasites

In epidemiology, transmission of pathogens is expected to proceed quicker in groups that are in close contact than through solitary individuals. Incidence and spread of fungal contamination increases in the huge egg masses of Simuliidae (Coupland 1992). Similarly, fungal disease spreads rapidly through winter aggregations of coccinellids and is considered to be the main biotic factor causing winter mortality (Hodek

et al. 1996). The Reduviidae whose faeces causes aggregations in shelters, often deposit the faeces just outside the shelter. This is thought to reduce the risk of disease spread, in particular against the flagellate *Blastocrithidia triatomae* (Lorenzo and Lazzari 1996).

Deteriorating environmental conditions

Especially in the egg masses, a deterioration of the physical environment has been reported. Near the centre of the egg mass, eggs frequently experience oxygen deprivation, which causes a delay in development and increased mortality (Imhof and Smith 1979, Kyorku and Raybould 1987). For plant feeders, communal attack might result in a stronger response of the chemical defence machinery of the plant (Rhoades 1985, Geervliet et al. 1998). Overexploitation by aphids can wilt plants, such that risky emigration to new host plants is necessary (Way 1973). In the stored product pest species, the increased humidity and metabolism in hot spots increased fungal contamination of resources and carbon dioxide build-up in the atmosphere, with potentially severe fitness consequences (Hardman 1977).

Increased conspicuousness to natural enemies

By forming an aggregate, the group as a whole is more conspicuous to natural enemies than a solitary individual would be. That might result in an aggregative or numerical response of natural enemies. A cue that is often used by predators is the aggregation pheromone itself, i.e. the natural enemy eavesdrop on the communication of their victims (Haynes and Birch 1985b, Dicke and Sabelis 1992, Stowe et al. 1995). In a number of families, the compounds that function as pheromone within the species, are used by natural enemies (as kairomone) in locating their victims (Harris and Todd 1980, Wood 1982, Aldrich et al. 1984, Moriya and Shiga 1984, Aldrich 1988, Aldrich et al. 1991, Wiskerke et al. 1993a, Yasuda and Tsurumachi 1995). Probably this exploitation by natural enemies occurs in many more families, since these few reports were strongly biassed towards studies in field set-ups, where the responses of natural enemies are immediately apparent, whereas in laboratory studies the natural enemy responses need to be investigated specifically. The strategy of intercepting behaviour by non-calling males has been suggested as a means of avoiding parasitism (Aldrich et al. 1984, Aldrich 1988). In the next section ('interspecific interactions'), both aspects of aggregation pheromones in predator-prey interactions will be shortly discussed from a population dynamical perspective.

Physiological costs

The investment of insects in producing aggregation pheromones must involve considerable physiological demands. The amount of pheromone that is produced is sometimes huge, and is often replenished during life. Sometimes, specialized glands and organs are involved, and these too have to be built and maintained. The insects that produce pheromones only after feeding, often via faeces, might avoid part of these costs. The lowered production of pheromones in Coleoptera in crowded situation (e.g., Pierce et al. 1989, Bartelt et al. 1993a, Bartelt and James 1994) could indicate that they use their pheromone resource sparsely (or conversely, avoid overcrowded situations).

Aggregation pheromones in interspecific interactions

Aggregation pheromones and natural enemies

As seen in the previous paragraphs, aggregation can be both beneficial and detrimental in terms of predator attack. For it to be beneficial, the *per capita* risk of mortality due to natural enemies must be lower in an aggregate than it is for a solitary individual. Reversely, by forming aggregations the members can become more conspicuous, either visually, or chemically because natural enemies use the pheromones for locating their victims (Dicke and Sabelis 1988, Roland 1990).

Theoretical papers on the effect of aggregation and density dependence in predator-prey interactions are numerous. Being in a herd, even without active defence, can reduce the *per capita* risk of mortality (Hamilton 1971). This could arise by reduced efficiency of natural enemies at high densities of their prey, due to handling time effects and, in parasitoids, re-encounters with already parasitized hosts (for discussion, see for example Hassell 2000). At the population level, randomly searching parasitoids lead to unstable population dynamics (Hassell and May 1973, 1974, May 1978). By aggregating the attack on high host densities, however, predator-prey population dynamics could potentially be stabilized, because the risk of parasitism becomes more heterogeneous (Chesson and Murdoch 1986, Ives 1992).

An example of the aggregative response of natural enemies to the density of prey is the predation by ladybird beetles (Coccinellidae) on aphids. The density dependent aggregative response arose because the individual predators remained significantly longer in larger colonies, and more importantly, large colonies were exploited by larger numbers of beetles and beetles remained longer in habitats with higher numbers of aphids (Ives et al. 1993). Such density dependent responses, that might be mediated by the prey's aggregation pheromone, can potentially nullify the benefits of a selfish herd effect (Hassell 1982).

Aggregation pheromones and community ecology

In many taxa, interspecific or cross attraction was reported, although most species preferred conspecifics when given the choice (see also 'similarities across taxa'). Asymmetric cross attraction (one species is attracted by another but not vice versa) was sometimes reported (Verhoef et al. 1977, Bartelt et al. 1986, Chambers et al. 1990). Such patterns could originate from on asymmetric reciprocal competitive impact, or they might reflect a derived character that allows only the derived species to respond to both mixtures (Bartelt et al. 1986, 1988), but see also (Schaner et al. 1989d).

Cross attraction increases interspecific interactions and could theoretically lead to competitive exclusion (Hardin, 1960): When multiple species compete for the same resources, only one species is predicted to persist on that resource. On the other hand, *intra*specific aggregation can reduce *inter*specific competition, relative to a random distribution of individuals: When individuals of different species cluster independently in parts of the environment, interspecific interactions are reduced relative to the intraspecific interactions. The parts of the environment that are not used by one species, can serve as refuges for others, facilitating coexistence and promoting biodiversity. This has been formalized as the aggregation model of coexistence (Atkinson and Shorrocks 1981, 1984, Ives 1988) and was shown for a

number of insect communities (Shorrocks et al. 1984, Kneidel 1985, Ives 1991, Shorrocks and Sevenster 1995, Sevenster and Alphen 1996, Toda et al. 1999, Wertheim et al. 2000).

Different strains of a species sometimes differ in the precise composition of their aggregation pheromones, mainly in the ratio of the components (Boake and Wade 1984, White et al. 1989) or in their responses to (combinations of) components (Bartelt et al. 1986). The speculation that this could eventually lead to reproductive isolation and speciation seems unlikely because of the low specificity for production of (Eller and Bartelt 1996) or response to a particular blend (White and Chambers 1989).

Aggregation pheromones and pest insects

Over half of the species that were studied for aggregation pheromones involved pest species. That is partly because most research on insects is biassed towards species that are interesting from an anthropocentric point of view. Yet partly it is also because it is the aggregating behaviour itself that makes them a pest. Additionally, not only their numbers, but also their frequent associations with rot, fermentation and pathogens gave them their pest status.

The aggregation pheromones are not only causing problems, but are also applied for pest management (Renwick 1992). Since many of the pest species attack human food crops, one has to be careful applying pesticides (Phillips 1994). There are several examples in which (aggregation) pheromones of insects are successfully used for mass trapping or monitoring (Howse et al. 1998, Suckling 2000).

Discussion

All living organisms use chemicals in their interactions and communication with other organisms. For humans it has not always been apparent, how prominent and ubiquitous this chemical signalling is. And even after the discovery of many extraordinary examples, we still often fail to recognise how important chemical communication is in the ecology of plants and animals. By neglecting the adaptive aspects of this mode of communication, it becomes extremely difficult to properly assess the ecological significance of the behaviours they induce and the interactions they mediate. Since aggregation has huge impacts on many aspects of ecology, this concerns a large area of research.

In this survey, we compared the mechanisms and functions of the use of aggregation pheromones across a large number of taxa. The occurrence of aggregation pheromones has evolved numerous times, and resulted in a diversity of mechanisms. Quite prominently, however, large similarities were also found across and within taxa. For example, the linkage between the possession of aggregation pheromones and the association with fungi and micro-organisms was strikingly apparent across a number of taxa. In general, the connection with food exploitation was frequent. Also the relationships with predator avoidance and defence, and the alterations of the micro-environment were manifest.

It is worthwhile to mention here, that the aggregated oviposition as a result of aggregation pheromones leads to a similar situation as the oviposition of large clutches. A number of taxa in butterflies (Lepidoptera) are known to cluster their eggs in large batches, and similar benefits were named as mentioned above, especially the increased efficiency in food exploitation (larvae), thermoregulation (eggs and larvae) and aposematic colouration (adults, eggs and larvae) (e.g., Stamp 1980). A recent comparative study on survival curves in gregarious vs solitary herbivorous caterpillars indicated a higher survival for the gregarious species. Remarkably, this could not be explained by possession of chemical defences, and was therefore attributed to either the 'quicker learning' hypothesis, dilution of risk or reduced exposure to predators through more rapid development (Hunter 2000). Thus, even within a group of highly related members, the evolution of aggregative behaviour cannot be explained simply by predator defences.

By some authors it was proposed that the aggregation pheromones are just an alternative or derivative of sex pheromones (e.g., Landolt 1997). This seems to be incorrect for almost all species. Other studies restrained functional explanations of aggregating to the hypothesis on predator avoidance or defence (Sillén-Tullberg and Leimar 1988, Hunter 2000). In reality, the implications of the use of aggregation pheromones for the individual are much wider, and sometimes not even related to any sexual function or predator interaction. Still, sexual interactions are obviously facilitated in many cases, simply because the two sexes are close together. This illustrates that it does injustice to assign a single function to the use of aggregation pheromones for an insect, as mostly they benefit organisms in many ways simultaneously. Each individual bears all advantages and disadvantages of being part of an aggregation, and for aggregation pheromones to persist, the benefits must outweigh the costs. Optimality and game theory approaches are needed to fully appreciate how all costs and benefits balance within the setting of the organism's ecology. Thus, the statement of Borden (1985) that aggregations serve for 'protection, reproduction, feeding or a combination thereof' seems the only befitting.

The evolution of the use of aggregation pheromone is under a constantly changing selection regime, due to the changing environment and (co-)evolution of resources, conspecifics and natural enemies. After the initial arising of a pheromone, the biology of the insect undergoes many changes due to natural selection. An advanced state of evolution can disguise the previous steps. Furthermore, evolution might be strongly directed to multi-functionality and thus parsimonious versatility of single natural products (Blum 1996). How exactly the aggregation pheromones initially arose is mostly unknown, and primary and secondary functions are difficult to assign. Hypotheses on primary functions have often invoked group selection arguments. Furthermore, significant profits are often only reached after reaching a certain group size, while significant costs at smaller group sizes might cause considerable evolutionary impediments. For example, in one bark beetle species as many as 40 galleries/m^2 were needed to overcome host resistence, while at lower densities females continuously had to clear liquid resin from their galleries, hardly fed and had to abandon or died within their galleries (Raffa and Berryman 1983). Kin selection and 'viscous systems' (i.e., no panmixis but greater interactions with direct neighbours) can create a starting point from which a chemical compound can evolve into an aggregation pheromone. Otherwise, the situations must be those where the initial costs of group living are relatively small, for example in the case

of high abundance or ephemerality of food, or where the compounds serve (initially) another function, as for example with sex pheromones, anti-aphrodiastics or defensive secretions, or when benefits are already achieved at very low densities.

The use of aggregation pheromones affects the individual insects as well as higher ecological and evolutionary processes. The shortage of integrated studies on ecological, functional and evolutionary aspects of the use of aggregation pheromones severely hampers our perception of the significance of this communication behaviour. Additionally, more field studies are indispensable, since the complexity of the communication by pheromones can only be fully elucidated by studying them in the ecological web of interactions. The existing extensive descriptions on mechanisms should now be accompanied by rigorous investigations on the functions. By comparing the different insect orders, general patterns emerged that might serve as a start to initialise further evolutionary and ecological investigations.

CHAPTER 3

The role of aggregation pheromones in an ecological web: A field study on *Drosophila*

Abstract

Signals in information conveyance have the potential to play a large role in the ecology of insects, because they often affect the behaviour of both conspecifics and heterospecifics. For our investigation into the ecological costs and benefits of the use of aggregation pheromone in the fruit fly *Drosophila melanogaster*, we explored which aspects of the ecology of these insects are affected by aggregation pheromone. In field experiments in the Netherlands and in France, we quantified the attractiveness of the pheromone and its effect on oviposition site selection and described the natural behaviour of fruit flies in aggregations. To study the effect of aggregation pheromone on interspecific interactions, we quantified the response of heterospecifics to the aggregation pheromone of *D. melanogaster* and *D. simulans* and describe the community from collected fruit samples. Fruit substrates with aggregation pheromone were significantly more attractive to adult *D. melanogaster* and *D. simulans* than control fruit substrates. The response to the pheromones was strong and positively dose dependent. Females deposited significantly more eggs on pheromone-treated fruits than on control fruits, and the micro-distribution of eggs within fruits was correlated to the micro-distribution of the pheromone. The extremely high densities of fruit flies in the large aggregates appeared to reduce the oviposition rate of females. Physical interactions with conspecifics and heterospecifics were frequently observed in the aggregations, and often led to patch leaving of the fruit flies. Competing species and natural enemies were also significantly attracted to the aggregation pheromone of *D. melanogaster* and *D. simulans*. Competition for food among larvae occurred at high densities and parasitism was density dependent. These results show that a multitude of aspects of the ecology of *Drosophila* is affected by the aggregation pheromones, both directly and indirectly. The importance of incorporating the communication signals in ecological theory is discussed.

Introduction

Many animals have an aggregated distribution in natural environments (Begon et al. 1996). This is partly due to the spatial nature of the environment, for example due to a localized distribution of resources and habitats that are suitable for survival and reproduction (i.e., spatial heterogeneity) (Begon et al. 1996) or due to an inherent effect of localized dispersal (Tilman et al. 1997) and localized competition (Lehman and Tilman 1997). Within the suitable region of the environment, however, aggregation is also frequently observed due to directed behaviour of individuals: Females lay their eggs in clutches or individuals actively form groups or aggregates during (part of) their lives. In the latter case, the individuals communicate and selectively choose those localities where conspecifics are present. Aggregative behaviour itself is recognized as important for many different aspects of the ecology of animals (Parrish and Edelstein-Keshet 1999), such as their micro-environment (e.g., Lockwood and Story 1986), interactions with natural enemies (e.g., Hassell and May 1974) and coexistence of species (e.g., Shorrocks et al. 1984, Toda et al. 1999, Wertheim et al. 2000). Conversely, the communication process, leading to the formation of animal aggregations, is as yet hardly acknowledged in ecological cost-benefit analyses of animal aggregations.

Individual organisms are embedded in large and complex ecological communities. Communication can provide a link between both conspecific and heterospecific individuals in those communities, and might therefore play a potent role in a variety of interactions. One important group of communication signals are those for chemical communication: the infochemicals (Dicke and Sabelis 1988). The use of infochemicals as the signals in communication evokes both ecological benefits and costs for its users. On the one hand, infochemicals have a high specificity and a large range of action, enabling the sender to influence the interactions with others at a relatively low energy expenditure (Baker 1985). On the other hand, after release of the infochemical, the signal is no longer under control of the sender, but is freely available for all with a personal interest. Infochemicals give information on the locality and 'identity' of the emitter, enabling espionage and misuse by conspecifics, natural enemies and competing species (Dicke and Sabelis 1992, Vet and Dicke 1992, Stowe et al. 1995, Haynes and Yeargan 1999).

Our interest is in a special group of infochemicals: the aggregation pheromones. Aggregation pheromones are substances, released by an individual, that induce conspecifics to cluster in certain localities of the environment (Shorey 1973, Borden 1985). Aggregation pheromones are widespread among insects and are found in many different orders and families (Shorey 1973, chapter 2). The benefits of using aggregation pheromones are not always clear, but must be sought in individual gains of joining or forming a group, outweighing the physiological and ecological costs of doing so (Pulliam and Caraco 1984, Borden 1985, Parrish and Edelstein-Keshet 1999). These benefits are often much broader than purely sexual (Shorey 1973, Borden 1985) and can also involve interspecific interactions (Shorey 1973, Borden 1985, chapter 2).

In many ecological studies concerning aggregations, the pheromones are overlooked as a generator in the behavioural patterns of the organisms under study. Yet, if we want to understand the behaviours that we observe, the dynamics within a population, or the interactions between different species, it is necessary to include the influence that pheromones have on individual behaviour and species interactions.

Both the aggregative behaviour itself *and* the aggregation pheromones could largely influence the interactions in the ecological web surrounding each individual.

In a field study, we explored the effects that aggregation pheromones have within an ecological community, both on individual behaviour and on interspecific interactions. We used fruit flies, *Drosophila* spp., as a model system, since many *Drosophila* species possess aggregation pheromones, and they are easily studied in the laboratory and the field. In *Drosophila* the aggregation pheromones are produced by males. During copulation, a large pheromone load is transferred to females. The pheromone is emitted by mature males in relatively small quantities, and by recently inseminated females in large quantities. In the laboratory, the aggregation pheromone is attractive to males and females when supplied in combination with feeding and oviposition substrate odours (e.g., Bartelt and Jackson 1984, Bartelt et al. 1985b, Schaner et al. 1987, Hedlund et al. 1996b). The only field study on aggregation pheromones of *Drosophila* demonstrated that application to substrates of the crude hexane extracts of males, containing the pheromone, significantly increased the combined numbers of four *Drosophila* species that were captured on these substrates (Jaenike et al. 1992). Attraction was stronger within species groups than between species groups, i.e., individuals were attracted stronger to substrates treated with the hexane extract of members of their own species group than to substrates treated with the hexane extracts of members of another species group. The spatial distribution of *Drosophila* larvae across substrates in nature is often aggregated (e.g., Rosewell et al. 1990, Sevenster and van Alphen 1996), but it is still disputed whether that results from large clutch sizes of single females or from aggregations of ovipositing females (Jaenike and James 1991, Shorrocks and Sevenster 1995, Sevenster 1996). The interspecific interactions with competitor species (other *Drosophila* and insect species) and natural enemies take place mostly during the larval stage. Food competition among larvae can be severe within a single substrate (Atkinson 1979, Grimaldi and Jeanike 1984). The most important natural enemies of *Drosophila* in temperate regions are larval parasitoids (Janssen et al. 1988). In the laboratory, some of these parasitoids, *Leptopilina* spp., were innately attracted to substrates with the aggregation pheromones of *Drosophila* (Wiskerke et al. 1993a, Hedlund et al. 1996a).

Based on this knowledge, we hypothesize that aggregation pheromones play an important role in the ecology of *Drosophila*, and a multitude of interactions could to be involved. In a field study in the Netherlands and France, we explored the effects of aggregation pheromone on a) the intraspecific attraction, b) oviposition site selection, c) the behaviour of fruit flies in the formation of aggregations and d) the interspecific interactions, using *D. melanogaster* and *D. simulans* as focal species. Since both species use the same compound as aggregation pheromone, and in the field they are hard to distinguish, they were treated as one 'combined' species in the behavioural assays. Our main questions for the experiments were based on our ongoing search for the ecological costs and benefits of aggregation pheromones in *Drosophila*. We addressed the following questions:

A. *Intraspecific attraction*: How strong is the behavioural response of adult flies to the aggregation pheromone, and how does it affect the distribution of adult flies across resources in a natural and complex environment? Is the response dose-dependent? By using the major active component

of the aggregation pheromone in synthetic form and the naturally deposited pheromone, we quantified the behavioural response to intraspecific signals in communication. We infer that strong responses of conspecifics indicate an important function of the aggregation pheromone in the ecology of these insects.

B. *Oviposition site selection:* Does the aggregation pheromone affect oviposition site selection by female flies? Since *Drosophila* larvae often have an aggregated distribution across resources and the pheromone is largely emitted by recently inseminated females, we hypothesize that one function of the pheromone actually is the aggregation of offspring.

C. *Behaviour in aggregations:* What happens in the natural formation of aggregations? By directly studying the behaviour of fruit flies on resources, and their interactions with both con- and heterospecifics, we try to identify selection pressures that shape the use of aggregation pheromone in *Drosophila*.

D. *Interspecific interactions:* What other species respond to the aggregation pheromone of *D. melanogaster* and *D. simulans*? And what are the interspecific interactions within the community? By characterizing the community to which the two focal species belong, and by assessing the response of heterospecifics to the aggregation pheromone, we identify the interspecific interactions that are affected by the use of aggregation pheromone. These interactions can result in both costs and benefits.

Materials & methods

Localities

The field experiments were performed during two years and in two localities: In Wageningen, the Netherlands (NL; mid July-September 1998, July-mid August 1999) and in Gotheron (Drôme), France (FR; mid August-mid October 1999). In the two localities we used a block of 20 (NL) or 24 (FR) apple trees in low maintenance orchards, where fruits were not harvested. To arrest a natural fruit fly population in the Dutch orchard, fruit waste from the fresh market was dropped weekly at the edge of the orchard, from May (1998) / June (1999) until the end of experiments; the pile was situated approximately 5 m from the experimental block. Because the density of wind blown fruit on the orchard floor was considerably higher in France (0-25 fruit/m^2) than in the Netherlands (0-6 apples/m^2), we did not need a fruit waste pile in the French field site. In the Netherlands, no other orchards were present in the direct vicinity (radius of 4 km), while in France the orchard was surrounded by other orchards, mainly containing peach trees. For the behavioural experiments in both countries, we did not use trees from the edge rows.

Substrates

Artificial and natural substrates were used in the experiments. The first was suited better for behavioural observations and the latter reflected better a natural situation.

The artificial substrate was an apple-yeast mixture (AY-mixture) of Golden Delicious apples, ground in a blender (approximately 1.5 dl water was added for 1.5 l mashed apples) and 6-8 hours before the start of the experiment it was mixed with dried bakers yeast (Engedura - Gist Brocades, 1998; Fermipan - Gist Brocades, 1999) in 100:1 w/w ratio, unless stated otherwise. The artificial substrate was offered to a natural fruit fly population, either in 'discs' with 50 micropatches (30 g of AY-mixture, spread out evenly over a PVC disc (14 cm ⌀, 1 cm height) with 50 holes (12 mm ⌀, 4 mm depth)) or in 'petri dishes' (a thin layer of 8 ml ≈8.5 g of mixture in petri dishes of 5.4 cm ⌀, 1.4 cm height or in lids of 175 ml Greiner containers, 5.0 cm ⌀, 2.0 cm height). In the petri dishes, 16 early second instar larvae were added to the thin layer of substrate to accommodate foraging parasitoids. Thirty minutes after adding the larvae, the petri dishes were used in the experiments. The 50-holes-discs allowed for a 'within-substrate' (microscale) variation in pheromone distribution, whereas in the petri dishes we could rear the larvae until adulthood.

The natural substrates were undamaged Golden Delicious apples collected from the orchard floor, in which two holes were punctured through the flesh (not touching the core) with the rear of a paintbrush (each hole approximately 1 cm ⌀, 3-5.5 cm depth, depending on the size of the apple). The two holes represented two natural micropatches, suitable for oviposition, and by treating only one of the holes we created a microdistribution of pheromone . The punctured apples were placed singly in transparent containers (11 cm ⌀, 8.5 cm height) with a layer of moist vermiculite. After collection from the field, the containers were covered with fine gauze and kept in a shelter to rear the insects. The temperature in the shelter fluctuated between 15 °C and 30 °C. The vermiculite was kept moist throughout the development of the insects.

Pheromone application

To obtain substrates with naturally deposited aggregation pheromones, 12 virgin females plus 12 virgin males (FM) of *D. melanogaster* were placed in a container with 4 petri dishes containing AY-mixture for 6 hours (matings were observed almost immediately). The control treatment consisted of placing 24 virgin *D. melanogaster* females (F) in a similar set-up. After removing the flies, all deposited eggs were removed with a pair of tweezers; the control patches were similarly disrupted with a another pair of tweezers.

The major active compound of the aggregation pheromones of both *D. melanogaster* and *D. simulans*, cis-Vaccenyl Acetate (Z-11-octadecenyl acetate), abbreviated as cVA (Bartelt et al. 1985b, Schaner et al. 1987), is commercially available (99% pure, Sigma Aldrich, PheroBank, Wageningen, the Netherlands). This compound dissolved in hexane is as attractive as the full natural blend in laboratory assays (Bartelt et al. 1985b, Schaner et al. 1987) and retains attractiveness for at least 3 days once applied to a substrate (Wiskerke et al. 1993b). Whenever the synthetic pheromone was used, cVA was diluted in n-hexane (*pro analysis*), and 15 μl of this dilution was pipetted onto the substrate (standard dilution of 4.5 μg cVA in 15 μl hexane, approximately 15 female fly equivalents (Bartelt et al. 1985b)). For the dose response studies, we also tested 0.45 μg cVA and 45.0 μg cVA in 15 μl hexane. The control treatment for synthetic pheromone was 15 μl n-hexane. In the pheromone-treated 50-holes-discs with AY-mixture, 5 of the 50 micropatches each received a standard application of 4.5 μg cVA; in the control discs 5 micropatches received a topical application of 15 μl hexane only. Immediately after the application,

the discs were used in the experiments. The petri dishes with AY-mixture were each given one standard application of cVA, or one standard application of hexane. Before adding the larvae (see above, 'substrates'), the hexane (both in treated and control petri dishes) was left to evaporate for 15-30 minutes. For the apples with two punctured micropatches, one of the micropatches was given either a standard application of synthetic pheromone or an application of hexane; the other micropatch was left untreated. The natural substrates were immediately used for experimentation.

Statistics

In most experiments, a set of several identically treated substrates were offered simultaneously to the fruit fly population. These experiments were generally repeated on 3 different days. We regarded the total number of identically treated substrates (i.e., generally 12) as the number of replications. It could be argued that the substrates that were offered simultaneously should be considered pseudo-replications, since they were simultaneously drawn from the same local population (Hurlbert 1984). In our opinion, however, the fruit fly population in the field was so large, that the chances of one substrate interfering with another substrate were sufficiently small to consider them as independently sampled. To allow for examination of the data per day, the data of the three days are also presented separately in the figures. The 3 days were included in the statistical tests as a block factor.

Our goal was to obtain quantitative models for the effect of pheromones on the behaviour of fruit flies. Since our data were mostly not normally distributed, we used a Generalized Linear Model (GLM) approach (SAS, v. 6.12: Proc Genmod). For comparison of numbers (e.g., counts of adults, eggs and the egg load of females), we specified a Poisson distribution with Log link function; for comparison of fractions (e.g., the microdistribution of eggs, percentage parasitism, see below), we selected a binomial distribution with Logit link function. Residue plots were checked for appropriateness of these specifications. Dscale was used to correct for overdispersion when deviance/df exceeded $1 \pm 2 \times \sqrt{(2/df)}$. The estimates of parameter values of the GLM for the counts were back transformed to obtain a 'factor of difference', which describes the ratio in numbers on the two substrate types ($N_{pheromone} / N_{control}$) that can be attributed to the presence of pheromones. The responses of males and females (intraspecific attraction) and the emergence of *D. melanogaster* and *D. simulans* (oviposition site selection) were compared by means of a 2 x 2 G-test with Williams correction (Sokal and Rohlf 1995) on the summed totals across all replications (Sokal and Rohlf 1995). The response of heterospecifics to the aggregation pheromone of *D. melanogaster* and *D. simulans* was evaluated with a G-test with Williams correction on the summed totals per species per country, under the null hypothesis of equal attractiveness of pheromone-treated and control substrates. The residence times on patches of male and female fruit flies were compared, using a Cox Regression Model. Below, some specific remarks on the statistics are added to the description of the experiments to provide more detail.

Experiments

A. Intraspecific attraction (NL and FR)

To test the attractiveness of aggregation pheromone in the field to *D. melanogaster* and *D. simulans*, a set of four pheromone-treated patches and four control patches (AY-mixture) was offered to a natural population of drosophilid fruit flies. The patches were placed on the ground of the orchard in two rows of four, at regular intervals in alternating order (2-3 m between rows and 2 m between patches in a row). Then the numbers, species and, if possible, sex of the fruit flies on each patch were recorded every 10 minutes for 2 - 4 hours (mainly depending on weather and day light conditions); presence of other insects was also recorded. Since patch residence times were frequently longer than 10 minutes, the counts represent a combination of chemotactic and arrestment responses to the aggregation pheromone, here considered together as the measure of attractiveness. To test the differential attractiveness of substrates with pheromones and control substrates, four variables were compared with a GLM: the numbers of fruit flies on the two substrate types (a) at the first count (10 minutes after starting the experiment), (b) at the 1 hour count, (c) at the 2 hour count, and (d) summed over the whole experimental period (cumulative totals). Each experiment was repeated at 3 different days. We tested:

A 1. Attractiveness of synthetic pheromones (NL and FR)

The attractiveness of synthetic pheromones was evaluated on the 50-holes-discs. In the Netherlands the experiments were started around mid-day. In France the experiments were done both in the morning (FR, 9.30 am) and in the evening (FR, 5.00 pm) to capture any diurnal effect. For the morning experiments in France, the apple-yeast mixture was prepared only 0.5-1 hour before the start of the experiment. For comparison with the experiments using petri dishes, we also tested synthetic pheromone using the petri dish set-up (see 'substrates'). To compare the responses of the two sexes, the data on synthetic pheromones in the Dutch experiments were pooled.

A 2. Attractiveness of naturally deposited pheromones (FR)

To acertain that naturally deposited aggregation pheromone gave similar results to the synthetic compounds, we offered petri dishes with AY-mixture that were incubated with *D. melanogaster* (see 'pheromone application'). Experiments started at 5.00 pm.

A 3. Dose response with synthetic pheromones (FR)

To establish the relationship between pheromone dose and response, a series of doses was applied to petri dishes with AY-mixture. The four doses were: (1) 45.0 μg cVA, (2) 4.5 μg cVA, (3) 0.45 μg cVA, all diluted in 15 μl hexane and (4) the control, 15 μl hexane only = 0 μg cVA. Of each treatment, 4 patches were offered simultaneously to the fruit fly population, thus a total of 16 patches was placed in the orchard each day. Experiments started at 5.00 pm. To derive quantitative models for the data of the dose response experiment, the dose was transformed (Ln (dose + 1)) and these transformed values were tested as linear and quadratic continuous predictors in the GLM.

for later identification. The following identification keys were used: *Drosophila* species (Bächli and Burla 1985), Diptera families (Oosterbroek 1981). The distinction between *D. melanogaster* and *D. simulans* could only be accurately made for the males; females of these species were assigned to the two species in proportion to the ratio in males. With GLM models, we tested for the relations between larval density and (1) rates of parasitism and (2) competition. Food competition among larvae was estimated by measuring the thorax length (de Moed et al. 1997, French et al. 1998) of 5 *D. melanogaster* males and 5 *D. melanogaster* and/or *D. simulans* females from each substrate.

Results

A. Intraspecific attraction (NL and FR)

In each of the field experiments on the attractiveness of aggregation pheromone (A1 to A4), the substrates treated with pheromone were significantly more attractive to adult *D. melanogaster* and *D. simulans* than the control substrates. Although the block factor 'day' was significant in all the experiments, the qualitative pattern was always the same and day * treatment interactions were mostly not significant.

A 1. Attractiveness of synthetic pheromones (NL and FR)

The numbers of *D. melanogaster* and *D. simulans* that were recorded on substrates with synthetic pheromone were consistently higher than those on the control substrates (fig. 1), as also demonstrated from the significant differences in the four variables that describe attractiveness (table 1). At the first count, ten minutes after offering the substrates to the fruit fly population, the differences between substrates were immediately apparent, with at least 4.5 times as many fruit flies on the pheromone-treated substrates than on the control substrates. Males were more selective than females in their response to pheromones, i.e., their total numbers had a higher ratio for pheromone-treated substrates to control substrates than female numbers had (total numbers in NL experiment: Males: 238 on cVA-treated discs vs 41 on hexane-treated discs; Females: 169 vs 71, 2 x 2 G-test: $p<0.001$).

The data in figure 1 depict the same experiment, but performed under three different conditions. The figure shows that the conditions influenced the observed densities of fruit flies, but not the qualitative overall preference for pheromone-treated substrates. In the Netherlands, the fruit flies were recorded in more or less constant low densities (fig. 1d). In France, the morning experiments show a sharp decline in recorded densities (fig. 1h), whereas the evening experiments show a steep increase in recorded densities (fig. 1l). The interval temperature measurements, taken during each experiment, demonstrate that densities dropped strongly at temperatures above 25 °C. Additionally, in the hour before nightfall, densities strongly increased. The experiment with petri dishes (figs. 2a-d; smaller AY-patches with less pheromone) attracted lower numbers than the 50-holes-discs (fig. 1i-l), but the preference for pheromone-treated substrates remained highly significant (table 1). Overall, densities of fruit flies were much higher in France than in the Netherlands, but both populations responded significantly to the presence of aggregation pheromone.

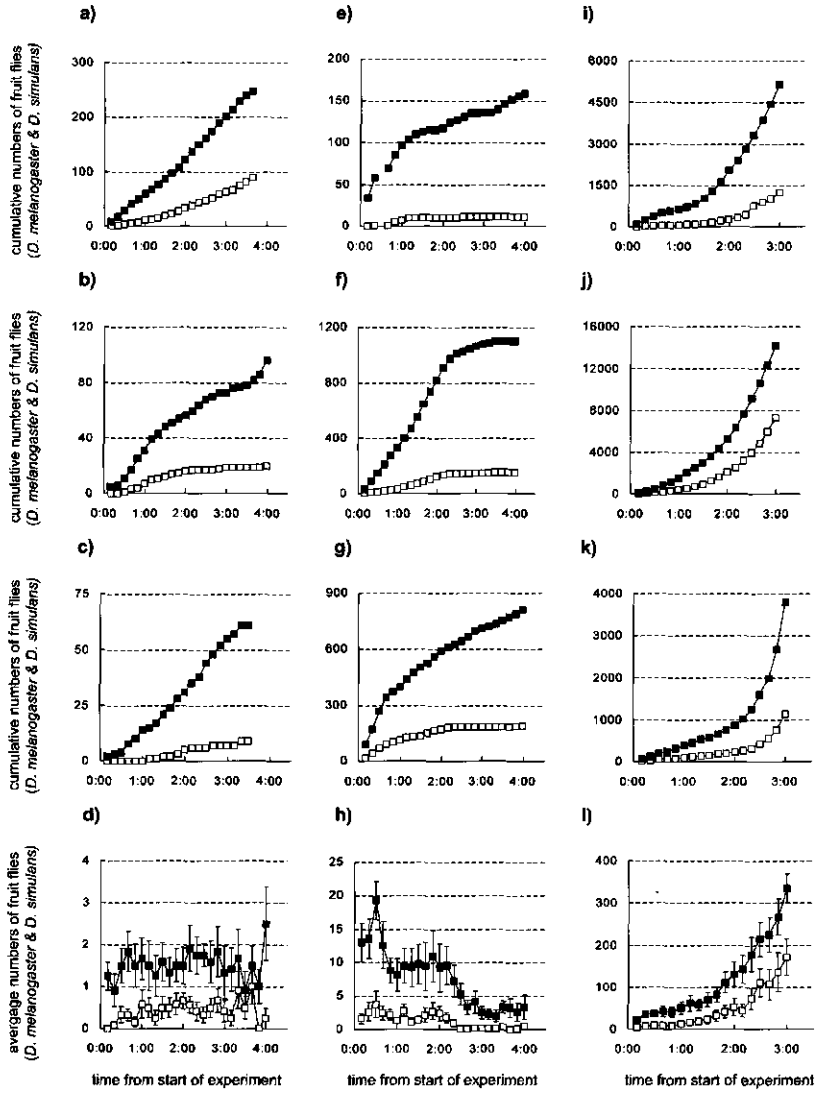

Figure 1: Numbers of fruit flies (*D. melanogaster* and *D. simulans*) on artificial substrates treated with synthetic pheromones (closed symbols, ■) and on control substrates treated with hexane (open symbols, □). The artificial substrates were offered in 50-holes-discs (see methods), and experiments were performed in the Netherlands, starting around mid-day (first column, **a–d**), and in France, starting around 9.30 am (second column, **e–h**) or starting around 5 pm (third column, **i–l**). The first 3 rows show the cumulative numbers of fruit flies on the 2 substrates types for each testing day separately, summed over the 4 discs per treatment. The last row shows the average numbers of fruit flies per substrate type (± standard errors), based on the 12 replications.

Table 1: The factors of difference (with 95 % confidence intervals) that describe attractiveness of substrates with pheromones compared to control substrates. For each experiment, four variables were compared in a Generalized Linear Model (GLM): the numbers of flies at the first count (10 minutes after starting the experiment), at the 1 hour count, at the 2 hours count and the cumulative numbers of flies. The factors are the back transformed parameter estimates of the GLM and show the ratio in the numbers of fruit flies relative to control substrates without pheromones, attributable to the presence of pheromone. A factor of 2 means a twofold increase in numbers, attributable to the presence of pheromone. For the dose response data, both a linear (L) and quadratic (Q) factor are derived for the model: Number = intercept + L*(Ln (dose +1)) + Q*(Ln (dose +1))^2. Doses range from 0 to 45 μg. Level of significance is denoted by ns ($p>0.05$), * ($p<0.05$), ** ($p<0.01$), or *** ($p<0.001$). 'D' indicates that standard errors have been adjusted to correct for overdispersion of the model.

experiment	Factors of Difference for the fly count at			
	10 minutes (95% CI)	1 hour (95% CI)	2 hour (95% CI)	cumulative (95% CI)
synthetic pheromone, disc, NL, mid-day	1E12 *** (1E12 -1E12)	2.86 * (1.21 - 6.77)	2.25 * (0.98 - 5.17)	3.24 *** D (2.04 - 5.74)
synthetic pheromone, disc, FR, 9.30 am	7.80 *** D (3.14 - 19.40)	6.13 *** D (2.29 - 16.41)	3.83 ** D (1.52 - 9.62)	5.83 *** D (3.14 - 10.80)
synthetic pheromone, disc, FR, 5 pm	4.48 *** D (2.03 - 9.88)	3.71 *** D (2.01 - 6.84)	2.49 *** D (1.64 - 3.78)	2.40 *** D (1.85 - 3.11)
synthetic pheromone, petri dish, FR, 5 pm	3.08 *** D (1.85 - 5.13)	2.10 ** D (1.27 - 3.48)	1.86 ** D (1.18 - 2.95)	1.81 *** D (1.36 - 2.42)
naturally deposited, petri dish, FR, 5 pm	1.61 * D (1.11 - 2.35)	ns	1.62 ** D (1.20 - 2.18)	1.51 *** D (1.23 - 1.87)
dose response, L: petri dish, FR, 5 pm	2.72 *** D (1.82 - 4.06)	1.75 ** D (1.22 - 2.49)	1.63 * D (1.12 - 2.38)	1.82 *** D (1.50 - 2.20)
Q:	0.83 *** D (0.76 - 0.91)	0.92 * D (0.84 - 1.00)	ns	0.92 *** D (0.88 - 0.96)
contamination test, petri dish, FR, 5 pm	4.58 *** D (2.50 - 8.37)	3.56 *** D (2.32 - 5.46)	3.89 *** (2.94 - 5.14)	3.34 *** D (2.84 - 3.93)

B. Oviposition site selection (FR)

B 1. Substrate selection

The fruit flies deposited over twice as many eggs on both the 50-holes-discs and apples treated with synthetic pheromones as on the control substrates treated with hexane (table 2, GLM, $p < 0.01$). On the discs, the numbers of eggs were positively related to the cumulative number of adults visiting the disc (fig. 3; GLM, significant terms for day, treatment, adult density and interaction between treatment * adult density). The significant interaction term describes a steeper positive relationship between the numbers of eggs and adult density on the hexane discs than on the pheromone treated discs, indicating a reduced oviposition rate in the large aggregations on the pheromone-treated substrates. The emerged numbers of *D. simulans* and *D. melanogaster* from the natural substrates did not indicate different selectiveness between the species for pheromone-treated substrates (the total numbers of emerged males: *D. simulans* 287 from cVA-treated apples vs 138 from hexane-treated apples; *D. melanogaster*: 810 vs 429, 2 x 2 G-test, ns).

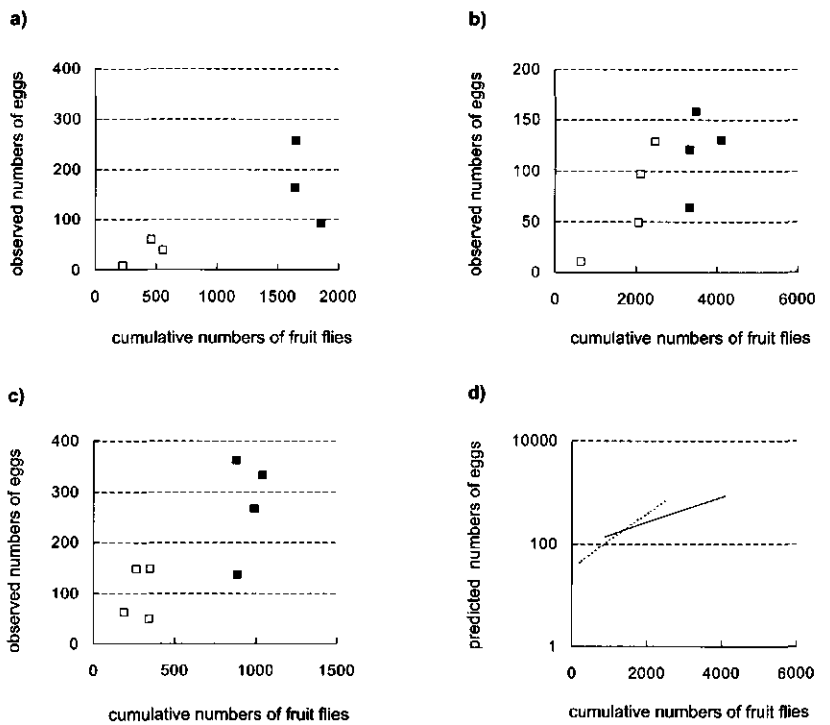

Figure 3: Relationship between the total numbers of fruit flies observed on substrates and the total numbers of eggs counted on these substrates. The total numbers of flies are based on counts every 10 minutes for 3 hours of observation on discs with artificial substrates (see methods) treated with synthetic pheromones (closed symbols, ■) and the control substrates treated with hexane (open symbols, □). a–c) The results are shown for each testing day separately. d) The numbers of eggs, as predicted by the statistical model for pheromone-treated (solid line) and control substrates(dotted line).

B 2. Microscale responses

Within resources, the micro-distribution of eggs also seemed to be affected by pheromones: We found a statistical trend ($p = 0.056$) toward larger fractions of eggs on the treated micropatches when the treatment was pheromone application than when it was hexane application (table 2).

B 3. Egg load

The egg load of mature females on pheromone-treated patches and control patches did not differ significantly (average egg load ± s.e. on cVA discs: 3.2 ± 0.42; on hexane discs: 2.3 ± 0.72, GLM: ns). Egg loads ranged between 0 and 12 and a further 0 – 19 nearly mature eggs were carried by the females (nearly mature eggs: average ± s.e. on cVA discs: 2.2 ± 0.42; on hexane discs: 3.0 ± 1.1). Also the ratios of mature and immature females were similar on the two substrate types (cVA discs: 63 mature vs 19 immature females; hexane discs: 15 mature vs 4 immature females, 2 x 2 G- test: ns).

similar numbers on the two substrate types. The non-drosophilids had mostly short patch residence times (less than 60 s) in which only substrate feeding was observed. Only the ants stayed for long periods of time (over 20 minutes) and they formed their own aggregations, also feeding on the apple-yeast mixture. The ants, Vespidae and callypterate flies physically interfered with the fruit flies, but they were seldomly seen to attack them. A negligible number of non-drosophilid eggs was observed in the behavioural assays.

D 2. Characterization of community (FR)

The insects that were reared from the collected apples and peaches were almost all Diptera, Hymenoptera and Coleoptera (table 3). The community was dominated by *D. melanogaster* and *D. simulans* and their larval parasitoids. The overall rate of parasitism by *Leptopilina* spp. on all *Drosophila* species was 16 % in peaches and 22 % in apples, but the percentage of fruit containing *Leptopilina* was considerably higher (71% of the peaches and 67 % of the apples). The risk of parasitism increased significantly with increasing larval density. The best fit for a GLM model on the fraction of parasitized larvae per collected resource was one that included date ($p<0.05$), resource type, i.e., randomly selected apples, punctured apples and peaches ($p<0.01$), density of larvae ($p<0.05$), a quadratic function for density of larvae ($p<0.05$) and interaction terms for density * resource type ($p<0.001$) and for the quadratic function of density * resource type ($p<0.001$). In this model, the relation between risk of parasitism and larval density is hump-shaped or saturating for the peaches and the punctured apples, and more or less constant or slightly increasing for randomly selected apples.

The thorax length, here used as an indicator for food competition among larvae, differed between the two sexes but showed for both sexes a hump-shaped pattern with increasing larval density (GLM, normal distribution with identity link, density was log transformed to homogenize variance), indicating that the largest body sizes were found at intermediate larval densities.

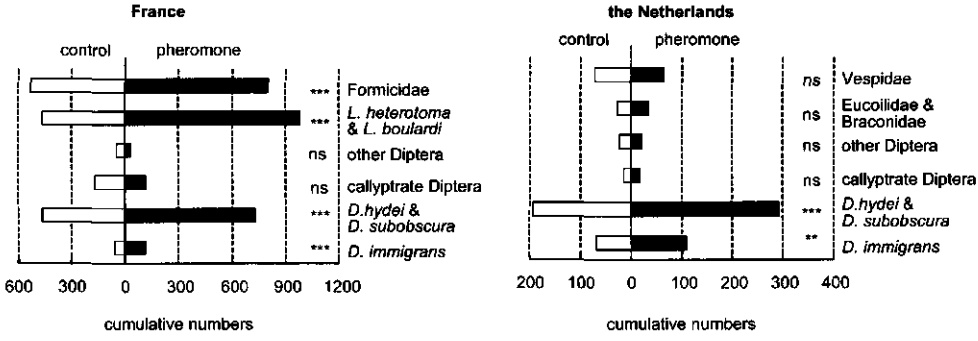

Figure 5: The response of other insect species to the aggregation pheromones of *D. melanogaster* and *D. simulans* in France **(a)** and in the Netherlands **(b)**. The values are the summed totals of all observations during all experiments (including 1 for which the *Drosophila* data was not shown). Comparisons were made with a G -test with Williams correction. Level of significance is denoted by ns (not significant, $p>0.05$), * ($p<0.05$), ** ($p<0.01$), or *** ($p<0.001$).

Table 3: Characterization of the frugivorous insect community in our French field site. Indicated are the total numbers of insects reared from the collected fruits. For more details, see methods.

	peach (n = 17)	apple – random (n = 51)	apple – punctured (n = 48)
Diptera			
Drosophilidae	4149	3972	3765
D. melanogaster Meigen	3608	3606	2888
D. simulans Sturtevant	523	328	742
D. immigrans Sturtevant	7	3	64
D. hydei Sturtevant	5	32	65
D. subobscura Collin	6	3	6
Muscidae	4	6	0
Scatopsidae	0	340	0
Cecidiomyiidae	3	4	0
Phoridae	0	0	2
Hymenoptera			
Figitidae	786	1243	908
L. boulardi (Barbotin et al)	432	1018	798
L. heterotoma (Thomson)	354	225	110
Pteromalidae	36	79	47
Pachycrepoideus vindemiae (Rondani)	34	73	46
Spalangia spp. Latreille	2	6	1
Braconidae			
Asobara tabida (Nees)	3	0	0
Coleoptera			
Nitulidae			
Carpophilus spp.	2	95	41
Staphilinidae	43	79	6
Coleoptera (others)	1	0	0
Others			
Collembola	2	14	2
Homoptera	1	1	3
Arachnidae	1	2	0

Discussion

Our field study shows that the aggregation pheromone plays a versatile role in the ecology of *D. melanogaster* and *D. simulans*, because it not only affects individual behaviour but also a variety of intra- and interspecific interactions. The ecological costs and benefits of using aggregation pheromone comprise both the aspects of aggregation and of communication. The extension of the ecological web with an information web realises intricate connections, that can alter the dynamics throughout the populations.

The behavioural response of adult *D. melanogaster* and *D. simulans* to the aggregation pheromone was immediate, robust and strong, both in the Netherlands and in France. The behavioural response was positively dose-dependent. Most importantly, the response to aggregation pheromone was prominent

under low and high adult and resource densities. It has been assumed that these pheromones evolved to overcome adverse conditions at low densities (i.e., an Allee effect) (Courchamp et al. 1999, Stephens and Sutherland 1999, Stephens et al. 1999) and therefore one could hypothesize that they should primarily act at low densities. This restriction was not found in our behavioural studies on adult flies: Under a variety of different conditions (e.g., adult density, density of alternative resources, climate, daytime) , the aggregation pheromone exerted a large influence on the *Drosophila* distribution. From the strength of the behavioural response, we deduce that the pheromone must be important for the fruit flies. Our hypothesis for a function in the aggregation of offspring is supported by our field data. Firstly, females oviposited over twice as many eggs on substrates with aggregation pheromone and even seemed to respond on a microscale distribution to the pheromone (within substrates). Secondly, those larvae that developed at intermediate densities in the collected fruit samples reached the largest adult size. For *Drosophila*, larger body size is generally associated with a higher fitness (Partridge et al. 1986, McCabe and Partridge 1997, Reeve et al. 2000). This hump-shaped relationship between larval density and body size would indeed suggest an Allee effect in the larval stage, but some caution is warranted: Our estimates of larval density were based on counts of survivors, thereby possibly confounding low larval density with high larval mortality.

As for the mechanism behind the aggregated distribution of larvae in the field (see introduction), we clearly show that the distribution of eggs is affected by aggregation pheromone, but we did not resolve whether that arises from large individual clutches or aggregations of ovipositing females. The mechanism of the aggregated distribution of larvae is of importance for models explaining coexistence of competitor species by spatial aggregation (Green 1986). These coexistence models have been applied to several *Drosophila* communities (Shorrocks et al. 1984, Jaenike and James 1991, Shorrocks and Sevenster 1995, Sevenster and van Alphen 1996, Toda et al. 1999, Wertheim et al. 2000) and show that intraspecific spatial aggregation results in unoccupied resources that serve as refuges for competitor species and thereby facilitate coexistence of many species. This theory is only explanatory, though, if aggregation of larvae originates from small clutches (Green 1986, 1988). Our field data show a low average eggload of females and our behavioural observations on ovipositing females also suggest small clutches. Furthermore, our data show that the pheromone induces the aggregation of females. These results favor the latter explanation, i.e., aggregations of ovipositing females, and would thus support one of the assumptions of the aggregation model of coexistence.

The aggregation pheromones have another serious implication for the coexistence of competitor species. The pheromone of *D. melanogaster* and *D. simulans* also attracted individuals of other *Drosophila* species (*D. immigrans*, *D. hydei* and *D. subobscura*). A similar result was found in the field study with hexane extracts of males in mycophagous *Drosophila* (Jaenike et al. 1992). The different fruit fly species that responded to the pheromones are considered to be competitor species. They all oviposit on the same substrates, and the larvae then compete for food. In our collected fruit samples, we found evidence for food competition among larvae, as indicated by a decrease in thorax length at high larval density. At the community level, strong association between competitor species could hinder coexistence (Sevenster 1996). The aggregation model of coexistence only applies when the distributions of competitor species are not tightly linked. In our description of the community, the weekly samples were too small to test for association between the competitor species, but other studies have shown consistent associations between

Drosophila species over years (Sevenster and van Alphen 1996, Toda et al. 1999). Nonetheless, in these studies intraspecific aggregation was sufficiently large relative to interspecific aggregation, thus facilitating coexistence. These results on association are also in accordance with the patterns found when the pheromones of two species groups were offered simultaneously to a natural population: individuals prefer those of their own species group (Jaenike et al. 1992).

In our study, the attraction of *D. immigrans* and *D. subobscura* to the aggregation pheromone of *D. melanogaster* and *D. simulans* could be (partly) attributed to the overlap in the pheromone components, as cVA is a component of the aggregation pheromone of each of the species (Bartelt et al. 1985b, Schaner et al. 1987, Hedlund et al. 1996b). This is, however, not the case for *D. hydei* (Moats et al. 1987). Although competitive outcome is not fixed between different species, but can depend on, for example, feeding rates of the larvae (Bakker 1961), trait*environment interactions (Sevenster and van Alphen 1993, Hedrick and King 1996) or priority effects ('who came first') (Shorrocks and Bingley 1994), it is intriguing that competitor species are preferring substrates with pheromones of their competitors above those without such odours, thus, associating rather than avoiding their competitors. Possibly, the observed cross attraction is adaptive, i.e., the individual could also gain some advantage from group forming with heterospecifics.

The attraction of natural enemies (*L. boulardi* and *L. heterotoma*) to the substrates with aggregation pheromones of *D. melanogaster* and *D. simulans* confirms the results of earlier laboratory experiments (Wiskerke et al. 1993a, Hedlund et al. 1996a): The use of aggregation pheromones by fruit flies could effectuate an ecological cost through increased conspicuousness of their offspring to natural enemies. By responding to the pheromones, the parasitoids can localise the substrates which have been visited by recently mated females, and thus increase their chances of finding hosts. Such eavesdropping on pheromonal communication was also reported for other parasitoids (e.g., Dicke and Sabelis 1992, Vet and Dicke 1992, Haynes and Yeargan 1999). On the other hand, aggregations can also function as 'selfish herds' (Hamilton 1971), where the per capita risk of attack is diluted with increasing density. We found some evidence for this in the decelerating risk of parasitism in the collected fruit samples. Further investigations will focus on the influence that the fruit fly aggregation pheromones have on the behaviour of the parasitoids and on risk of parasitism in host aggregations (chapters 6 and 7).

The consequences of using aggregation pheromone comprise both direct effects (e.g., increased attractiveness of substrates to both conspecifics and heterospecifics) and indirect effects (e.g., increased interference due to increased adult densities). Both can largely influence the behaviour of individuals, but direct effects are rarely investigated in ecological studies. Typically, aggregation pheromones are studied more from a mechanistic or applied perspective (e.g., chemical identification, applications for pest management) and they are rarely integrated in ecological theory. In contrast, aggregation itself is studied more from a functional or population dynamical perspective, without considering the mechanism behind it. The importance, however, of linking the two is paramount. This is also illustrated in the field data on encounter and dilution effects in a predator-prey interaction (Wrona and Dixon 1991). This study showed that although individual prey in an aggregation experience a diluted risk of predation, at the same time encounter rates by predators increased, because the groups were apparently more conspicuous. The

positive effect of grouping was thus largely swamped by its negative effects, altering the net outcome of an ecological cost-benefit analysis. In our system, with a natural enemy responding to the aggregation pheromone of its victim (Wiskerke et al. 1993a, this study), it would be deceptive not to incorporate the response of the parasitoid to the pheromone of its host.

The ecological costs and benefits of using aggregation pheromones have to be sought on many different levels, according to the multitude of effects that they can cause. For this, both the indirect and the direct effects have to be considered. It is likely that these costs and benefits will differ for males, females and offspring. Our field study suggested that at least the following costs could apply: Increased interference between adults, increased competition for mates, reduced oviposition rates, increased conspicuousness of larvae to parasitoids/natural enemies, and increased competition for food among larvae. The functions or benefits of the use of aggregation pheromones are not immediately apparent from our data, but they too could apply to the adults, eggs and larvae since all are aggregated. Further studies are needed to determine whether the benefits are associated with reducing the risk of predation (of adults, eggs or larvae), larval resource exploitation, altering the micro-climate, or a combination of those (chapter 2).

Acknowledgements

Mr. Marbouti, Vincent Mercier and Freddy Combe are gratefully acknowledged for allowing the field experiments on the premisses of the INRA in Gotheron, and for providing accommodation and research facilities. The research group of Michel Boulétreau in Lyon is thanked for valuable discussion and kind hospitality. Gerrit Gort guided the statistical analyses. Yde Jongema helped with the identification of the insects. The Entomology PhD discussion group gave valuable comments on the manuscript. Grants by the SIR-NWO, PICS and the Uyttenboogaart-Eliasen Foundation enabled the field study in France.

CHAPTER 4

Behavioural plasticity in support of a benefit of using aggregation pheromone in *Drosophila melanogaster*

Abstract

Plasticity in traits can evolve in response to environmental variability and enables an individual to adopt the optimal strategy under different conditions. Interactions between organisms are highly variable, and behavioural plasticity can reflect an altered cost-benefit balance in the biotic interaction. We explored behavioural plasticity in the use of aggregation pheromone in the fruit fly *Drosophila melanogaster* Meigen. Based on previous field observations, we formulated two hypotheses on a benefit of using aggregation pheromone for aggregated oviposition. One hypothesis concerns a benefit to the females themselves, where reduced harassment by males can enhance oviposition rate; the other concerns a benefit to their offspring, where larvae can exploit arduous substrates more efficiently. We derive contrasting expectations on the strength of the behavioural response to pheromone for substrates that differ in nutritional quality to larvae. High quality substrates relax the strength of larval competition, which allows for stronger aggregative responses of the females, but conversely, it may yield aggregation less necessary when the benefit is related to resource exploitation by the larvae. In indoor and outdoor dual choice set-ups, we tested the behavioural responses of the adults to the aggregation pheromone with substrates of varying quality, and examined oviposition behaviour. The response of adults to the aggregation pheromone was strong and robust for low quality substrates, but significantly weaker for a high quality substrate. This supports the hypothesis on a benefit to the larvae. Females retained aggregation pheromones in the absence of oviposition substrates for at least 24 hours. In the outdoor set-up, substrates with aggregation pheromone received more than three times as many eggs as control substrates, and this was directly related to the number of adults that visited each substrate. Per capita oviposition rates were not different for differently sized aggregations, and consequently, no evidence was found in support of the hypothesis on reduced harassment. The connection between the possession of aggregation pheromones and a mutualistic relationship with micro-organisms for *Drosophila* and other insects is discussed.

Introduction

Plasticity in traits evolves in response to environmental variability (Via and Lande 1985, Vet et al. 1990, Via et al. 1995, Karban et al. 1999). The costs and benefits that are associated with a trait vary between environments, and consequently, so does the optimal strategy. To maximize the fitness in each environment, it can be adaptive when an organism adjusts its phenotype (i.e., its physiology, morphology and/or behaviour) in response to the prevailing cost-benefit balance. The variability in the environment can have an abiotic origin (e.g., light, temperature, nitrogen supply), but also biotic interactions can induce phenotypic responses (e.g., Dobson 1989, Dudley and Schmitt 1996, Agrawal and Rutter 1998). Interactions between organisms are inevitably variable, which provides scope for natural selection to promote plasticity (Thompson 1988). A change in the characteristics of the interaction can cause immediate responses or induce physiological and morphological changes. The most immediate phenotypic adjustment, in response to an altered cost-benefit balance, is a change in behaviour. Therefore, the study of behavioural plasticity can provide a valuable tool in identifying costs and benefits of biotic interactions.

Signalling is a behavioural trait that mediates the interaction between organisms. Signalling between animals implies that under certain circumstances it is beneficial to call upon others. For example, enticing mates can require the announcement of one's willingness, or resource exploitation can be more efficient by combining forces. Obviously, signalling also comprises costs, most importantly because the signals can be exploited by everyone in the food web (Dicke and Sabelis 1992, Dicke and van Loon 2000). For instance, when an organism produces a signal to attract a mate, natural enemies or competitors can also use it to their own benefit. Signalling and responding to signals is likely to occur when the expected benefits exceed the expected costs. Whether the benefits outweigh the costs can differ between situations.

Many drosophilid fruit fly species possess aggregation pheromones (Bartelt and Jackson 1984, Bartelt et al. 1985b, 1986, 1988, 1989, Moats et al. 1987, Schaner et al. 1987, Schaner et al. 1989a, b, c, d, Jaenike et al. 1992, Schaner and Jackson 1992, Hedlund et al. 1996b). In *Drosophila*, the aggregation pheromone is produced by the males, and transferred to females during copulation. Both males and recently mated females release the pheromone and attract other males and females to a substrate. On such substrates (e.g., fermenting fruit and sap streams, mushrooms, rotting plant material), the adults feed, mate and oviposit (Spieth 1974). A recent field experiment on frugivorous *Drosophila* showed that the pheromone induced aggregated oviposition: Resources with aggregation pheromone received at least twice as many eggs as resources without aggregation pheromone (chapter 3). The hatched larvae feed on the yeasts that develop on the resource (Brito Da Cunha et al. 1951, Dudgeon 1954, Cooper 1959, Begon 1986). Larvae at high densities can experience severe competition for food, causing a longer developmental period, increased larval mortality and a reduced adult body size and fertility (Sang 1956, Bakker 1961, Atkinson 1979, Grimaldi and Jeanike 1984). Thus, the use of aggregation pheromone for aggregated ovipositing evokes a clear cost in terms of increased larval competition at higher aggregation densities.

The benefits for using aggregation pheromone are different for male and female fruit flies. Males can attract potential mates. Females can only acquire the pheromone through mating, after which they

become unreceptive to other males (Manning 1962). These mated females do, however, still respond to the pheromone, by selectively choosing those substrates that harbour the pheromone (chapter 3). This suggests that also for mated females the pheromone yields a benefit, and this benefit is not related to mate finding. Our field observation on the increased number of eggs on pheromone-treated substrates (chapter 3) indicates that a benefit can arise from aggregated oviposition. Two hypotheses emerged from our field experiments: 1) Aggregated oviposition is beneficial to the adult females, because it shields them from harassment by males. Our behavioural observations revealed that females are continuously disturbed during feeding and ovipositing by courting males (chapter 3), and clustering might shield them somewhat from this, as was found for aggregating female dragonflies (Martens and Rehfeldt 1989); 2) Aggregated oviposition is beneficial for larval growth and survival. The insects that were reared from natural substrates were smaller when they emerged in low densities from natural substrates than those at intermediate densities (chapter 3), which is in agreement with previous reports that larval resource exploitation is more efficient in groups (Sang 1956, Ashburner 1989). We will explore these two hypotheses by studying the plasticity in the behavioural responses of the adult fruit flies to aggregation pheromone.

When the main benefit of clustered ovipositing is to the females, because it shields them from harassment by males, we would expect that the increased clustering of offspring comprises an associated cost. In that case, the higher the larval resource quality, the stronger the response to the pheromone can be. Furthermore, oviposition rates should increase with increasing adult densities. Reversely, when the main benefit is to the offspring, because larval development is more prosperous after aggregated ovipositing (through reduced fungal growth and/or increased yeast development, see chapter 4), we might expect that enhanced larval substrate quality reduces the need for aggregation. In that case, the higher the larval resource quality, the weaker the response to the pheromone can be.

Another aspect of plasticity is the emission of pheromone by mated females. The females were reported to deplete the majority of pheromone within 6 hours after mating (Bartelt et al. 1985b), while oviposition is initiated at the earliest two hours after mating (B. Wertheim, unpublished results) and can continue for several days after one single mating (Chapman et al. 1996). The females can only acquire aggregation pheromone through mating, while mating is a costly process in terms of survival (Chapman et al. 1995). Thus, the limited supply of pheromone should be used sparsely if the deposition of pheromone is adaptive for females in enhancing communal oviposition. Therefore, we would expect that females deprived of oviposition substrate would retain at least some pheromone until they have access to an oviposition substrate.

To test these predictions, we used dual-choice bio-assays in which we offered pheromone-treated substrates and control substrates. In an indoor set-up, we tested the chemotactic response of adults to the aggregation pheromone, varied the nutritional quality of test substrates to examine the effect on the response to the aggregation pheromone, and tested whether females retained aggregation pheromone in the absence of oviposition substrates. In an outdoor set-up, we examined the effect of aggregation pheromone on oviposition rates in differently sized aggregations.

Materials & methods

Insects

The culture of *Drosophila melanogaster* Meigen originated from wild strain individuals reared from apples, collected in an orchard in Wageningen, the Netherlands, in 1995. The insects were subsequently reared on a medium of yeast, sugar and agar (40 g, 135 g resp 23 g in 1 l. of water) at 20 ± 1 °C and 16:8 L:D. Adult populations were kept in cages (30 x 30 x 40 cm) under the same climatic conditions.

Experimental set-up

Two set-ups were used for dual-choice bio-assays: Indoor flight cages and outdoor population cages. In both set-ups, pheromone-treated and control substrates were offered simultaneously to a *D. melanogaster* population, and the behavioural responses toward both substrate types were compared. The indoor set-up allowed for quick evaluation of chemotactic responses, whereas the outdoor set-up gave more comprehensive information on the behavioural responses and oviposition rates of the fruit flies.

The indoor flight cage was similar in design to the one used for earlier aggregation pheromone studies in *Drosophila* (Bartelt and Jackson 1984) and was developed and described in detail by Van der Sommen and co-workers (2000). Shortly, a flight cage (30 x 40 x 60 cm) with gauze side walls was stocked with 400 - 600 adult *D. melanogaster*. In this flight cage, 4 pheromone-treated and 4 control substrates were offered in trapping devices, and the number of flies that entered the traps within 4 minutes was recorded as a measure of the chemotactic response of the flies. A gentle wind stream (0.20 m/s) was generated with a fan, positioned behind two metal screens to allow for the development of odour plumes. The experiments were performed in a fume hood to avoid contamination of the air with pheromone. The traps were constructed from plastic vials (2 cm ⌀, 4 cm height) with a rim of parafilm around the opening, leaving an entrance hole of approximately 0.5 cm^2. The flies quickly entered these traps, but were restrained from leaving because of the parafilm rim. After the bio-assay, the trapped flies were discarded from the test population, and the remaining (naive) population in the flight cage was offered another set of traps, until 4 tests per day, spread out across the day. Each combination of substrates was tested 4 - 6 times with at least 2 different fly populations.

An outdoor population cage (3 x 6 x 2 m) was constructed of a fine gauze net, that was tied underneath two adjacent party tents (3 x 3 x 2 m each). This construction was put up on a stretch of grassland in the open air. The sidewalls of the tent were secured on the grassland with stones. In the outdoor population cage, we tested the chemotactic and arrestment responses with synthetic pheromone and after the behavioural assays, the numbers of eggs were recorded to test for effects of the adult density on female oviposition rates. For each experiment, 4 control standard substrates and 4 pheromone - treated standard substrates were placed on the ground in 2 rows (1 m apart), treatment and control substrates alternating (1 m apart). After placing the standard substrates on the grass, 400 - 800 *D. melanogaster* (sex ratio 1:1) were released by placing their holding pots horizontally on the floor in between the two rows, 1 m from the first two discs, and removing the plug. Typically, relatively large numbers of flies remained in their holding pots, and these were shaken out after 1 hour and 15 minutes ('second release'). From the

moment of the first release, the numbers of fruit flies on each standard substrate were counted every 5 minutes for 2 hours. These counts combined chemotactic and arrestment responses. The experiment was repeated on 3 days.

Standard substrates

The standard substrates that were used in experiments consisted of mashed apple, 'A', (Golden Delicious, mashed in blender with 1.5 dl water added for each litre of pulp, frozen upon preparation and defrosted before experimentation), or mashed apple mixed with living bakers yeast (Engedura, Gist Brocades) in specified w/w ratio ('AY'). To test for the influence of substrate quality on the response to pheromone, the mashed apple was either heat-sterilized at 120 °C, fresh (i.e., just defrosted) or incubated at room temperature for 24 hours ('1 day'), and we used apple-yeast mixture in ratio 150/1 or 15/1 w/w (mashed apple/yeast) to obtain a more explicit difference in substrate quality. In the indoor flight cages, 1 - 3 ml substrate was placed at the bottom of the trap vials. In the outdoor population cage, 30 ml substrate was spread out across a disc with 50 micropatches (disc: 14 cm ⌀, 1 cm height, PVC; micropatches: holes of 12 mm ⌀, 4 mm depth, each filled to the rim with substrate).

Aggregation pheromone

To treat substrates with naturally deposited aggregation pheromone, the substrates were incubated with *D. melanogaster*. Eight standard substrates (trap vials, without the parafilm rim) were incubated in a container (12 x 25 x 10 cm) for 16 hours with either 12 virgin females plus 12 virgin males (FM), 24 virgin males (M) or 24 recently mated females (F*). The control treatments consisted of a similar incubation with 24 virgin females (F) or without flies (0).

To test whether mated females retained aggregation pheromone in the absence of oviposition substrate, recently mated females were stored at 20 °C for 24 hours in vials without oviposition substrate, and thereafter they were incubated as described above (F*24).

We also tested with the synthetic pheromone of *D. melanogaster*. The major active compound, Z-11-octadecenyl acetate, common name cis-vaccenyl acetate (cVA) (Bartelt et al. 1985b), is commercially available (> 99 % pure, Sigma Aldrich, Pherobank, Wageningen, the Netherlands). The synthetic pheromone cVA was diluted in n-hexane (*pro analysis*), and a standard application consisted of 4.5 μg cVA in 15 μl hexane, which is approximately the equivalent of deposition by 15 recently mated females (Bartelt et al. 1985b). The control treatment consisted of 15 μl hexane. Synthetic pheromone and the control were pipetted onto standard substrates. For the indoor flight cage experiments, 1 standard application was added to each trap. For the population cage experiments, the standard pheromone or hexane applications were pipetted onto 5 micropatches per disc.

Statistics

For the indoor flight cage set up, the numbers of flies in the pheromone-treated and control traps were compared with a G-test, adjusted with Williams correction for a better approximation of the χ^2-distribution, and an extrinsic null hypothesis of equal attractiveness (Sokal and Rohlf 1995). The data were pooled for all replications per test combination. Differences between sexes and between substrate

types (separately for the mashed apple substrates [sterilized *vs* fresh *vs* 1 day old] and for apple yeast mixtures [AY 150:1 *vs* AY 15:1]) were tested with an R x C G-test with Williams correction (Sokal and Rohlf 1995).

For the outdoor population cages, the numbers of flies on the pheromone-treated and control substrates were compared at the first count, at the one hour and two hour count and for the cumulative totals (counts summed for the whole experimental period) with Generalized Linear Models (GLM, Sas v. 6.1, Proc Genmod, Poisson distribution and Log link function). Similarly, the numbers of eggs on the substrates were compared, with the cumulative numbers of fruit flies included in the analysis as a co-variable. The Factor of Difference (FoD, with 95 % Confidence Intervals) describes the increase in numbers on pheromone-treated substrates compared to control substrates ($N_{pheromone}/N_{control}$), attributable to the presence of pheromone. The sex ratio was compared for pheromone-treated and control discs, with density of adult flies and distance from the release point included as co-variables (GLM, Binomial distribution, Logit link function). The oviposition rate, calculated as the number of eggs divided by the cumulative number of flies, was regressed against adult density (GLM, Normal distribution, identity link function). Each disc was treated as a separate replication.

Results

In the dual choice experiments in indoor flight cages, significantly more *D. melanogaster* chose for pheromone-treated substrates than for control substrates (fig. 1). This applied both for synthetic pheromone (cVA *vs* hexane, G-test, $p<0.001$), and naturally deposited pheromone (FM *vs* 0, G-test, $p<0.001$; M *vs* 0, G-test, $p<0.001$). Substrates incubated with virgin female flies (that do not possess aggregation pheromone) were as attractive as control substrates (F *vs* 0, G-test, $p>0.05$).

Substrate quality affected both the numbers of flies that responded (fig. 2a) and their selectiveness for pheromone-treated substrates in relation to substrate quality (fig. 2b). For all different substrate types,

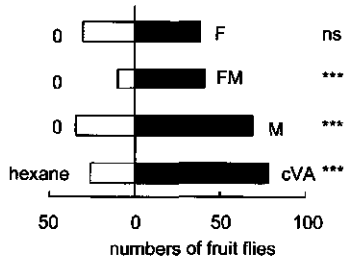

Figure 1: The numbers of *D. melanogaster* that chose for treated (solid bars) and control substrates (open bars) in dual-choice indoor flight cages. Substrates had been incubated with females plus males (FM), males (M), virgin females (F) or no flies (0). The first two substrate types (FM and M) contain aggregation pheromone. Alternatively, substrates were treated with the synthetic pheromone (cVA) or the solvent (hexane). Significance is denoted by: ns ($p>0.05$), *** ($p<0.001$).

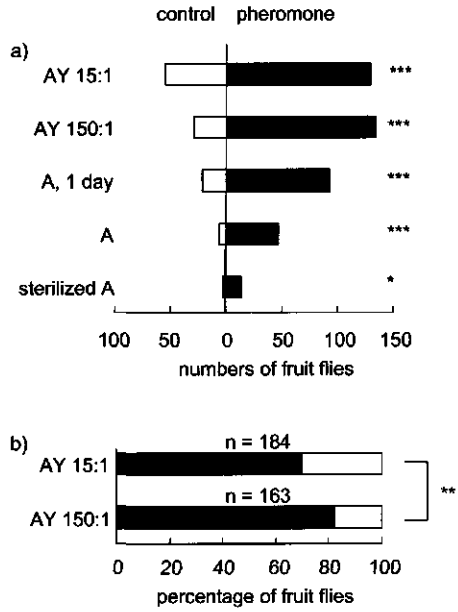

Figure 2: The influence of substrate quality on the responsiveness of *D. melanogaster* to synthetic aggregation pheromone. Mashed apple substrate (A) was manipulated to create a range of yeast concentrations: heat-sterilized, fresh (A), incubated for 24 hours at 20 °C (1 day), or mixed with living bakers yeast (AY) in specified w/w ratio. **a)** The numbers and **b)** percentages of flies that chose for pheromone-treated (cVA, solid bars) and control substrates (hexane, open bars) in dual-choice indoor flight cages. Significance is denoted by: ns ($p>0.05$), * ($p<0.05$) and *** ($p<0.001$).

significantly more flies chose for the pheromone-treated ones than for control substrates (fig. 2a: G-test, $p<0.05$ on sterilized mashed apple; $p<0.001$ for all other substrate types). In the mashed apple substrates the responsiveness was robust (>80% of the flies to the pheromone treatment) (3 x 2 G-test, $p>0.05$). In the apple-yeast mixture, however, a significant lower proportion of flies chose for pheromone-treated substrates when tested on substrates with high yeast concentration than on substrates with low yeast concentration (fig. 2b: G-test, $p<0.01$). Males and females did not differ in selectiveness (2 x 2 G-test, not significant for any experiment).

Mated females still possessed and deposited aggregation pheromone 24 hours after mating (fig. 3: F*24 *vs* 0, $p<0.001$). The enhanced attractiveness of test substrates incubated with these females was not significantly different from those incubated with recently mated females (2 x 2 G-test, $p>0.05$).

In the outdoor population cages, the numbers of flies were immediately and consistently higher on pheromone-treated substrates than on control substrates (fig. 4a). The four comparisons were all highly significant: More flies were present on the pheromone-treated substrates than on the control substrates at the first count (GLM, FoD = 4.0, CI = 2.3 - 7.1, $p<0.001$), after 1 hour (GLM, FoD = 3.2, CI = 1.9 - 5.2, $p<0.001$), 2 hours (GLM, FoD = 2.4, CI = 1.6 - 3.7, $p<0.001$) and summed over the

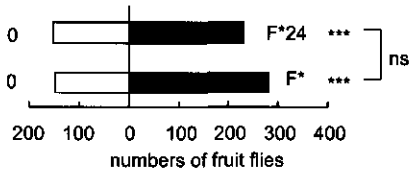

Figure 3: Possession of aggregation pheromone by mated females. Mated females were either incubated directly after breaking copula (F*) or first stored for 24 hours at 20 °C (F*24). Possession was indicated by different numbers of fruit flies that chose for treated (solid bars) and control substrates ('0', no flies, open bars) in dual-choice indoor flight cages. Significance is denoted by: ns ($p>0.05$) and *** ($p<0.001$).

whole period (GLM, FoD = 2.9, CI = 1.9 - 4.6, $p<0.001$). At first, the numbers of flies per substrate quickly built up, subsequently followed by a much more gradual increase. The second release of flies gave similar responses as the first, with again a quick accumulation and a subsequent more gradual phase. These stationary periods indicate that after the initial choice for a substrate, the fruit flies mostly remained on that substrate for the period of the experiment. The numbers of flies on the substrates were negatively related to the distance from the release point (GLM, $p<0.001$). The sex ratio was neither different on pheromone-treated and hexane-treated substrates, nor for different densities of flies or distances from the release point (GLM, $p>0.05$).

More than three times as many eggs were deposited on the pheromone-treated substrates (fig. 4b, GLM, FoD = 3.4, CI = 1.7 - 6.7, $p<0.001$). The cumulative number of fruit flies was a significant co-variable (GLM, $p<0.001$). When the outlier in the control substrate was included in the statistical analysis, the pheromone treatment * co-variable term was significant, but without this outlier it was not. The oviposition rate, calculated as the number of eggs divided by the cumulative number of flies, was not significantly different at different densities of flies (GLM: $p>0.05$).

Discussion

The plasticity in the behavioural response of female *D. melanogaster* supports the hypothesis of a benefit for aggregated oviposition to their offspring. We predicted that when aggregated oviposition yields a benefit in larval resource exploitation, enhanced larval substrate quality might reduce the need for aggregation. The response to the pheromone in our experiments was significantly lower in a substrate with a high concentration of yeast added, whereas the response was robust across low quality substrates. The behavioural plasticity in the response of females as an indication for a benefit of aggregated oviposition to their offspring receives rigorous support from experiments where we tested for positive effects of increased larval and adult density on larval development and showed that aggregated oviposition can indeed significantly enhance larval survival and growth (chapter 5). The present study illustrates that behavioural responses to conspecifics may be adjusted in relation to the prevailing conditions, and studying behavioural plasticity might provide a means to explore alternative hypotheses (Prokopy and Roitberg 2001).

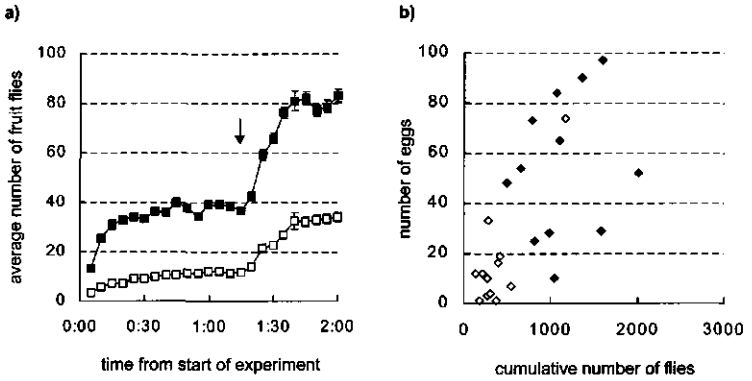

Figure 4: The behavioural responses of *D. melanogaster* to pheromone-treated (solid markers) and control substrates (open markers) in outdoor population cages. The two substrate types were offered simultaneously and the numbers of flies were counted every 5 minutes for 2 hours. Afterwards the number of eggs on each substrate was counted. **a)** The average numbers (± standard errors) of fruit flies during the behavioural assay. The arrow denotes the time of release for a second group of fruit flies. **b)** The number of eggs deposited on each test-substrate during the behavioural assays versus the cumulative number of flies that had visited the substrate.

We found no evidence for the hypothesis on a benefit in aggregated ovipositing for females through reduced harassment by males. In the population cages, the oviposition rate was not different in differently sized aggregations. Although significantly more eggs were deposited on pheromone- treated substrates than on control substrates, this difference was linearly related to the numbers of flies on the substrates. The population cage set-up ensured that the fruit flies could exhibit their natural behaviours on resources, including the interactions between males and females, while the influences of disturbance by other insects was absent.

Females were still able to deposit pheromone 24 hours after mating, although they were previously reported to deplete the majority of pheromone within 6 hours (Bartelt et al. 1985b). This indicates that they retained at least part of the aggregation pheromone for future use. Foremost, this means that any benefit of emitting pheromone for mated females is not restricted to the very short period after mating, but can be exploited for an extensive period of time. Furthermore, it might suggest that females are capable of controlling the emission of pheromone. If the emission of pheromone yields a benefit to them, this opens up a wide array of possibilities for studying plasticity as a tool to gain insight in costs and benefits of signalling. For example, females might deposit less pheromone when large numbers of females are around and save on their limited pheromone supply, or forego emission when substrate quality is sufficient to support the development of her own offspring while risk of competition is high. The variability in costs and benefits that are associated with the use of signals can result in adaptive behavioural plasticity.

The response to aggregation pheromone in our experiments was influenced by the concentration of yeast in the substrate. Previous studies already showed that substrate odours and aggregation pheromones are

synergistic, and the response of fruit flies to their aggregation pheromone alone is essentially nil (Bartelt et al. 1985b, 1988, Schaner et al. 1987, Schaner et al. 1989a, c, d, Van der Sommen et al. 2000). Furthermore, drosophilid fruit flies are attracted to specific odours that are produced during microbial degradation of resources (Fuyama 1976, Oakeshott et al. 1989). The adults are vectors for yeasts and bacteria to the breeding and feeding substrates, and within insect-infested resources, micro-organisms develop more quickly (Gilbert 1980, Fogleman and Foster 1989). This all indicates that *Drosophila* flies are closely associated with micro-organisms.

A strikingly large proportion of the many insect species that possess aggregation pheromones have similar associations with micro-organisms, developing on their feeding substrates (chapter 2). The most apparent example of such a relationship is the one between bark beetles and fungi. The sex in bark beetles that possesses the aggregation pheromone also carries fungi in specialized mycangia, and these fungi can be essential to overcome the defences of the beetles' host trees (Wood 1982). Furthermore, the fungi convert the often indigestible wood into readily available nutrients for the beetles and their larvae, and both feed on the mycelium (Kok 1979). Similar interactions, albeit often less specialized, have been reported for quite a large number of insects possessing aggregation pheromone. Behavioural plasticity in relation to microbial activity is also manifested in these insects. Often, the responses to the pheromone strongly increase, or are even only apparent when the pheromone is accompanied by odours produced by the micro-organisms (e.g., Borden et al. 1979, Wood 1982, Bartelt et al. 1992, Hallett et al. 1995, chapter 2). Possibly, the evolution of aggregation pheromones is somehow facilitated or even directed by the interaction with the micro-organisms. Alternatively, the interaction with micro-organisms could have evolved in response to the occurrence of aggregation pheromone in the insects. Both scenarios strongly indicate that the micro-organisms benefit from an aggregated vector, and the insects in their turn benefit from microbial growth. If such a mutual benefit indeed exists, we should find a positive relationship between insect density and microbial growth, and a further positive relationship with insect resource exploitation. Moreover, we should find evidence for it from the plasticity in behavioural responses of the insects to the aggregation pheromone, as we have shown for *Drosophila melanogaster* in this paper.

Whether the behavioural plasticity that we observed is adaptive depends on the full array of costs and benefits, that shapes the use of aggregation pheromone. In this paper, we focussed on benefits related to aggregated oviposition, and potential costs that arise from larval competition at large aggregation size. Many other costs and benefits can apply simultaneously. For example, the use of cues for intraspecific communication creates a situation that can be (mis)used by other species in the food web. The variability in the strength and frequency of such interactions determines whether plastic responses by the flies are favoured. As such, we can exploit behavioural variability in controlled bio-assays, and use it to generate hypotheses for further research.

Acknowledgements

Anton van der Sommen is thanked for developing the flight cages. Willy Herder and Anja van Aggelen helped in designing and constructing the population cage. Henk Snellen en Leo Koopman reared the insects.

CHAPTER 5

Allee effect in larval resource exploitation in *Drosophila*: An interaction between density of adults, larvae and micro-organisms

Abstract

Aggregation pheromones can evolve when individuals benefit from clustering. Such a situation can arise with an Allee effect, i.e., a positive relationship between individual fitness and density of conspecifics. Can we identify an Allee effect in the larval resource exploitation by *Drosophila melanogaster*, that could explain the evolution of aggregation pheromone in this species? Aggregation pheromone in *Drosophila* induces aggregated ovipositing in females. We hypothesize that an Allee effect in *D. melanogaster* larvae arises from an increased efficiency of a group of larvae to temper fungal growth on their feeding substrate. To test this hypothesis, standard apple substrates were infested with specified numbers of larvae, and their survival and development was monitored. A potential beneficial effect of the presence of adult flies was also investigated by incubating a varying number of adults on the substrate before introducing the larvae. Adults are known to inoculate substrates with yeast, on which the larvae feed. Fungal growth was negatively related to larval survival and the size of the emerging flies. Although the fungal growth on the substrate was largely reduced at increased larval densities, our measurements on fitness components indicated no Allee effect between larval densities and larval fitness, but larval competition instead. However, increased adult densities on the substrates prior to larval development yielded higher survival of the larvae, larger emerging flies and also reduced fungal growth on the substrates, hence adults enhanced the quality of the larval substrate. Thus, we showed significant benefits for aggregated oviposition in adult female fruit flies, through enhanced larval development. The aggregation pheromone itself did not directly contribute to the quality of the larval resource, as indicated by experiments with synthetic pheromone. The interaction between adults, micro-organisms and larval growth is discussed in relation to the consequences on total fitness.

Introduction

An aggregation pheromone is a substance, emitted by an individual, that induces aggregative behaviour in conspecifics (Shorey 1973). These pheromones have evolved several times in insects (chapter 2), which implies that under certain circumstances, selection can favour individuals that seek out conspecifics. Although a number of advantages is apparent for individuals in large groups (see for example Pulliam and Caraco 1984, Parrish and Edelstein-Keshet 1999, chapter 2), the evolution of aggregative behaviour requires that already at low densities, advantages outweigh all associated costs. Such a situation could arise when survival or reproduction is hampered at low population densities, i.e., with an Allee effect.

Recently, Stephens and co-workers (1999) provided a clear definition for the Allee effect: "A positive relationship between any component of individual fitness and either numbers or density of conspecifics". This definition emphasizes that the Allee effect is based on an advantage to the individual, and it accepts a wide range of mechanisms to achieve such benefits (for examples, see Courchamp et al. 1999, Stephens and Sutherland 1999). Additionally, a distinction was made between 'component Allee effects', manifesting positive density dependence on a component of fitness, and 'demographic Allee effects', concerning total fitness. The latter mostly depicts the hump-shaped relationship that is commonly associated with Allee effects, where at some density the positive density dependence dominates, but at other densities costs outweigh the benefits. The component Allee effect reflects only the isolated mechanism that yields a benefit to aggregation, which might or might not be sufficient to compensate for the connected costs.

If we expect for a specific species, that the evolution of an aggregation pheromone was driven by positive density dependence at low densities, we can start searching for a component Allee effect. In controlled experiments, the fitness consequences of isolated mechanisms can be measured, and might provide insight into the selective force that has promoted the use of aggregation pheromone.

The fruit fly *Drosophila melanogaster* Meigen uses an aggregation pheromone that is emitted by males and mated females (Bartelt et al. 1985b, chapter 3). In a previous field study, we identified potential costs and benefits to its use in this fly species (chapter 3). The adult flies aggregate, feed and breed at decaying fruits (Spieth 1974). The pheromone induces aggregated oviposition in females (chapter 3). Consequently, the larvae also have an aggregated distribution across resource substrates. The larvae of *D. melanogaster* feed on yeasts that develop on a substrate (Brito Da Cunha et al. 1951, Cooper 1959, Begon 1986), but at low larval densities, fungi and moulds can overtake a substrate instead (Ashburner 1989). In laboratory cultures on artificial substrates, larvae suffer high mortality at low larval density (Sang 1956, Ashburner 1989).

The aim of the present investigation is to identify a component Allee effect in resource exploitation by *D. melanogaster* larvae, that might explain the evolution of the use of aggregation pheromone in this species. We hypothesize that an Allee effect in *D. melanogaster* larvae can arise from an increased efficiency of a group of larvae to temper fungal growth. However, a recent study with larvae of *D. subobscura* feeding at a natural resource (rowan berries) showed no indication for any facilitation

when feeding in groups (Hoffmeister and Rohlfs 2001). Possibly, the type of substrate that the larvae develop on determines the need for aggregated distributions. Besides, larval density by itself might be insufficient to yield a benefit regarding resource exploitation. In fact, it is likely that adults contribute to the quality of the larval substrates, since a number of *Drosophila* species are known to inoculate substrates with different species of yeast (Gilbert 1980, Fogleman and Foster 1989, Morais et al. 1995).

To test for an Allee effect by larval densities, and a potential added role for adult flies, standard substrates were manually infested with specified numbers of larvae, or standard substrates were infested through oviposition by adult flies. The standard substrates consisted of mashed apple, which constitutes a fairly benign resource for larval development, or of apple chunks, which is both more natural and more demanding for larvae to exploit (B. Wertheim, personal observation). The observations during this first experiment inspired a more thorough procedure for a second experiment. Before manually infesting the standard substrates with specified numbers of larvae, each standard substrate received a preliminary treatment, consisting of incubation with a specified number of adult flies of specified sex, or the addition of the synthetic aggregation pheromone or its solvent. The fungal growth on the substrates was recorded throughout the larval development. The survival, developmental time and thorax length of the emerging flies were compared across larval densities and preliminary treatments.

Materials and methods

Insects

The culture of *D. melanogaster* originated from wild strain individuals reared from apples, collected in an orchard in Wageningen, the Netherlands, in 1995. The insects were subsequently reared on an agar gel medium of 40 g yeast, 135 g sugar and 23 g agar in 1 l of water and 8 ml nipagin solution added, at 20 ± 2 °C and 16:8 L:D. Adult populations were kept in cages (40 x 30 x 30 cm) under the same climatic conditions.

For the experiments, larvae and adults were isolated. To obtain isolated larvae, adults were allowed to oviposit on water agar (20 g agar in 1 l of water) with a thin layer of living baker's yeast (Fermipan, Gist Brocades, 250 mg / ml water). The eggs were rinsed from the yeast with water, and after one additional thorough rinse, transferred to petri dishes with water agar, and incubated at 25 ± 2 °C. Within three hours after hatching, the larvae were introduced to the standard substrates for the experiments (see below). To obtain isolated adult flies, pupae were rinsed from the rearing medium, transferred to vials with water agar, and separated by sex 1 – 2 days prior to emergence (the male sex combes on the front tarsi are visible through the pupal case). The emerged adults were kept for 3 – 5 days in vials with water agar (8 ml nipagin solution added per litre during preparation) before experimentation, and were provided with honey on a strip of filter paper.

Standard substrates for experiments

Two types of substrates were used in the experiments: Mashed apples and apple chunks. Mashed apple was prepared from Golden Delicious apples, ground in a blender with 1.5 dl water added for each litre of pulp. The pulp was frozen upon preparation, and defrosted at room temperature before use. Each standard substrate consisted of 8 ml of the pulp in a petri dish with ridges at the lid to allow some ventilation (5 cm ⌀; experiment 1) or in a 175 ml container with a ventilation hole, plugged with cotton wool (Greiner, 5 cm ⌀; experiment 2). The apple chunks (11 ± 2 g (experiment 1) or 10 ± 0.5 g (experiment 2)) were cut from Golden Delicious apples, such that one side was covered with skin. In one of the unskinned sides, an indentation of 1 cm was pressed with a glass rod (1 cm ⌀) to create a bruised spot where the first instar larvae could initiate feeding. Each chunk was placed in a glass vial (8 cm height, 4 cm ⌀) with a layer of moist vermiculite, and the vials were plugged with cotton wool.

Experiment 1:

To create series of substrates with different larval densities, a specified number of isolated larvae (N = 1, 2, 4, 8, 16, 32) was introduced to a standard substrate (mashed apple or apple chunk), using a small brush. Alternatively, mashed apple substrates were offered to an adult population for oviposition during different periods of time. In the latter case, the resulting eggs were counted to represent the larval density, assuming no egg mortality. The standard substrates were incubated in a climatized room at 20 ± 2 °C and 16:8 L:D. The development of fungal growth was categorized (0 - 10, 25, 50, 75 and 100% of the substrate surface) at 2 - 4 day intervals during larval development, and emerged adults were counted to calculate the percentage survival. In total, 10 - 20 replicate series were prepared for each of the three treatments. The replicate series that were prepared on the same day were grouped in blocks.

Experiment 2:

Standard substrates were treated prior to the introduction of a specified number of isolated larvae (N = 0, 1, 4, 8, 24). The preliminary treatments were according to an incomplete factorial design (see table 1), and consisted of incubation with adult fruit flies in specified numbers of specified sex, or the addition of synthetic aggregation pheromone (cis-vaccenyl acetate, 99 % pure, Ipo Pherobank, the Netherlands, 4.5 μg dissolved in 15 μl hexane) or the solvent (15 μl hexane). For the incubation of substrates with adults, adult flies were released in a container (12 x 25 x 10 cm) with 5 mashed apple substrates that would later constitute a series of larval densities. The number of flies that was released per container was 0, 5, 20 or 40 to obtain an average of 0, 1, 4 or 8 flies per substrate, or 0 or 4 adults were released in each vial with an apple chunk. After an incubation for 16 - 18 hours in a climatized room at 21 ± 1 °C, the adults were removed from the substrates. Before introducing the isolated larvae, we removed the eggs that were deposited by the virgin females on the mashed apple substrates with a pair of tweezers; the other mashed apple substrates were also disrupted with pairs of tweezers. After introduction of the larvae, the standard substrates were incubated in a glasshouse at 20 ± 3 °C at ambient spring light conditions. Several series of larval densities for each treatment were prepared at four different days (block factor), resulting in 10 - 17 replicate series per treatment in total.

Table 1: The preliminary treatments for the standard substrates in experiment 2.

abbreviation	standard substrate	preliminary treatment	Factorial design			replications (n)
			adults	sex	pheromone	
MA / 0F	mashed apple	no adults	0	-	no	17
MA / 1F	mashed apple	1 virgin female	1	female	no	17
MA / 4F	mashed apple	4 virgin females	4	female	no	17
MA / 8F	mashed apple	8 virgin females	8	female	no	17
MA / 4M	mashed apple	4 virgin males	4	male	yes	16
MA / cva	mashed apple	synthetic pheromone	0	-	yes	16
MA / hex	mashed apple	hexane (solvent)	0	-	no	16
CH / 0F	apple chunks	no adults	0		no	10
CH / 4F	apple chunks	4 virgin females	4		no	10

The percentage of the substrate that was covered with fungal growth was recorded at day 1, 3, 5, 7, 10 and 12 after introducing the larvae. The emerged adults were counted and collected daily from day 9 until day 13 and at 16 days after introducing the larvae, and stored in ethanol. The thorax length of the emerged flies was measured from the base of the most anterior humeral bristle to the posterior tip of the scutellum (French et al. 1998) under a stereo microscope with an eyepiece micrometer. To analyse the differences in developmental time and in thorax length of the emerged flies (experiment 2), the average values per standard substrate were calculated (separately for male and female thorax length).

Statistics

The data were analysed with generalized linear models (GLM, SAS, v. 6.1, Proc Genmod, with dscale option to correct for overdispersion when the scaled deviance exceeded a value of 1). For the percentage of substrate covered with fungal growth and the percentage survival, we specified a binomial distribution with logit link function. For the averaged developmental time and averaged male and female thorax lengths per standard substrate, we specified a normal distribution with identity link. We separately analysed the data for the different types of standard substrates (mashed apples and apple chunks), the isolated larvae and oviposited eggs (experiment 1), and the data for experiment 1 and 2. The block factors were included in all analyses. The factors that were tested were: number of larvae, number of adults, sex of the adults (nested), quadratic and third order terms of numbers of larvae and adults and pheromone presence (see also table 1). The number of larvae was log-transformed (Ln(larvae+1)), to avoid strong leverage for the high host density. The results for the minimal adequate models (MAM) are presented. The MAM was obtained by backward elimination of all those factors from the full model, for which removal caused an insignificant increase in deviance (F-test), starting at higher order terms (Crawley 1993). The degree of fungal cover was subsequently added to the MAM to test for an added explanatory value of the fungal growth, above and beyond the other factors.

Results

experiment 1:

In mashed apple, the larvae experienced competition rather than an Allee effect. The survival of larvae was negatively density dependent (first, second and third order terms: $p<0.001$). At first sight, the density dependent pattern between the treatment with eggs oviposited by flies (fig. 1a) and the treatment with manually infested larvae (fig. 1b) appeared to be different, with a hump-shape in the latter, suggesting an Allee effect. The statistical models, however, described monotonically declining survival rates for both cases, indicating competition. Nonetheless, the observation prompted us to closer examine the effect of adults on substrates in relation to larval development. In the apple chunks, survival was not density dependent ($p>0.05$; fig. 1c). The development of fungi (mainly *Mucor* spp., *Penicillium* spp. and *Aspergillus* spp.) was strongly reduced at high larval density in all different treatments (first order term: $p<0.001$).

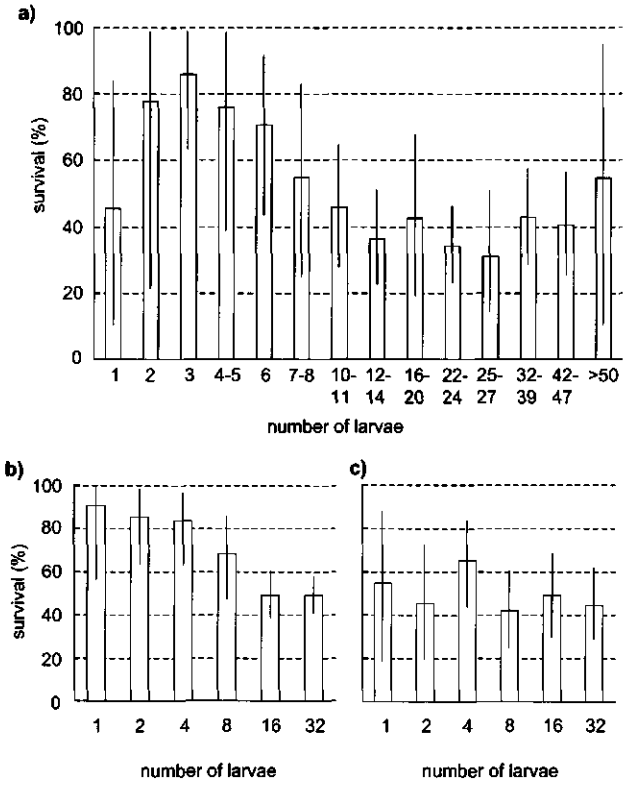

Figure 1: The survival of *D. melanogaster* larvae at different larval densities in standard substrates (experiment 1). The different densities were obtained by **(a)** offering mashed apple substrate patches to adult fruit flies for varying amounts of time or **(b)** by manually introducing specified numbers of isolated larvae on mashed apple substrates or **(c)** on apple chunks. See methods for explanation. The average percentage survival and 95 % confidence intervals (error bars) were calculated on angular transformed data and back -transformed.

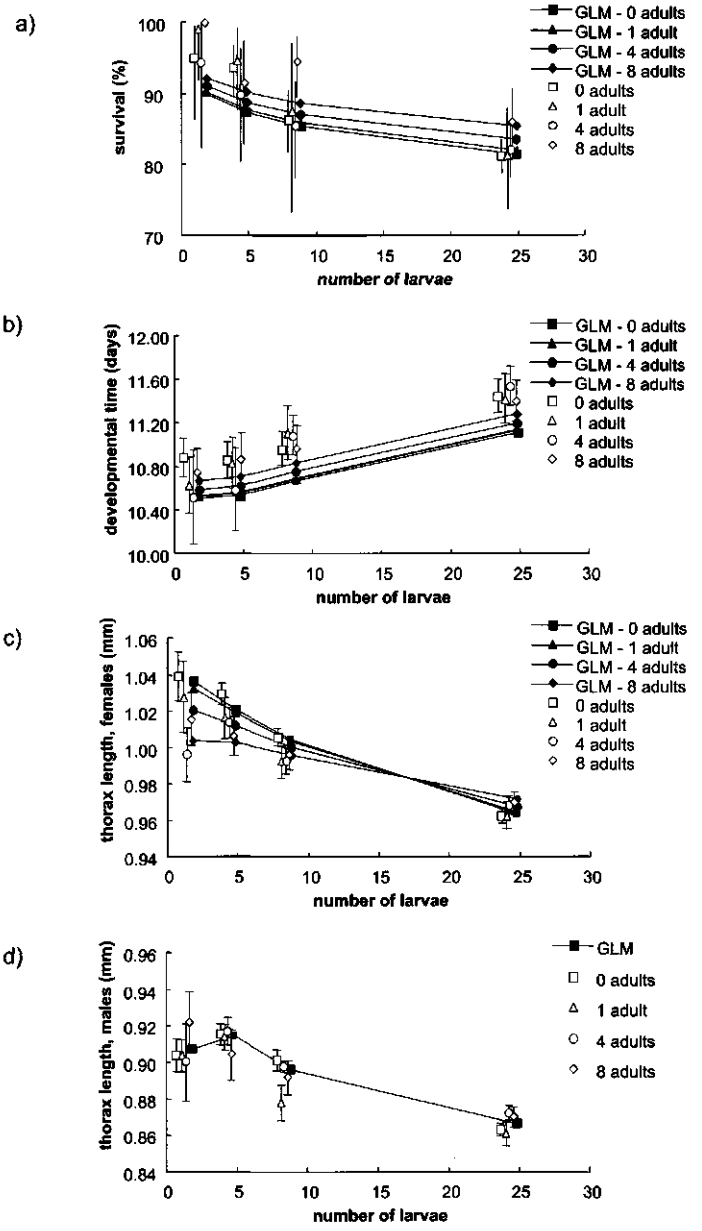

Figure 2: Average fitness components for larvae, reared at different densities in mashed apple standard substrates, which had been incubated with varying densities of adults, prior to the introduction of the larvae (see methods). **a)** The survival (+ 95 % confidence intervals) and **b)** developmental time (± sem.) of *D. melanogaster* larvae and the thorax length (± sem.) of emerging **c)** females and **d)** males. The open symbols depict the averages from our data; the closed symbols depict the minimal adequate model, as calculated with Generalized Linear Models (GLM). For clarity, the different series are slightly shifted around the value of x (larval density).

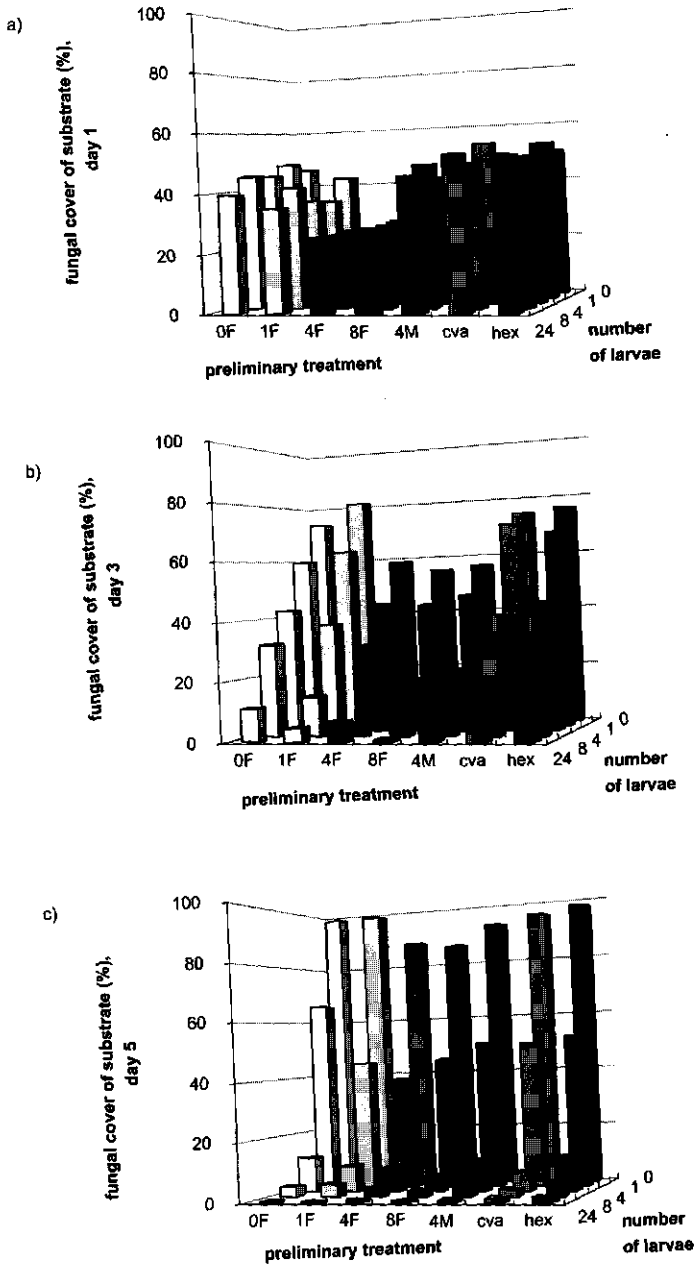

Figure 3: Fungal growth on mashed apple standard substrates with different preliminary treatments and different numbers of larvae. The average percentages of substrate that was covered with fungi were calculated on angular transformed data and back-transformed

experiment 2:

In mashed apple, again, we did not find an Allee effect for larval density on any fitness component. However, we did find a significant Allee effect for adult density on larval survival. The survival of the larvae was positively related to the numbers of adults that were incubated on the substrates, prior to the introduction of larvae (fig. 2a, first order term: $p<0.05$). For larval density, we found only negative relations with fitness components, indicating strong competition. Increased larval density reduced survival, (fig. 2a, first order term: $p<0.001$), prolonged averaged developmental time (fig. 2b, first and second order terms: $p<0.001$), and reduced the thorax length of emerging females (fig. 2c, second order term: $p<0.001$). The density of adults ($p<0.01$) and the interaction term between larval and adult density ($p<0.05$) were significant in the statistical model for female thorax length, indicating a negative relation between adult density and size at low larval density, and a positive relation at high larval density. The thorax length of males described a slight hump-shaped relation to larval density (fig. 2d, first and second order terms $p<0.001$, and third order term $p<0.05$). Adult density did not contribute to the explanatory power of this model. At higher adult densities, developmental period was slightly increased (first order term: $p<0.05$).

The development of fungi on the mashed apple was influenced by the density of larvae, and by the density and sex of adults during the preliminary treatments. Increased larval and adult densities strongly reduced the percentage of substrate that was covered with fungi (for larvae: first, second and third order terms, $p<0.001$ from the third day onwards; for adults: first and second order terms, $p<0.001$ resp. $p<0.05$ during the first 5 days; see fig. 3). Incubation with virgin females reduced fungal growth more strongly than incubation with virgin males ($p<0.05$ on day 1 and day 7, $p=0.055$ on day 3). Survival of the larvae was, above and beyond the larval and adult effects, negatively related to the degree of fungal cover on the substrate during the early development of larvae ($p<0.001$, fungal cover at day 3). After the pupation of larvae (approximately from day 6), fungal growth increased and short developmental times correlated with a higher degree of fungal cover during late larval development (from day 7 onwards, $p<0.001$). The degree of fungal cover during very early development had a slight positive relationship with female thorax length (at day 1, $p<0.05$). The presence of pheromone did not contribute to explanatory power in any of the described statistical models ($p>0.05$).

In the apple chunks, we again found no Allee effect for larval density, but significant Allee effects for adult density on larval fitness components. Both the survival (fig. 4a, $p<0.01$) and thorax length of emerging adults (females: fig. 4c, $p<0.001$; males fig. 4d, $p<0.01$) were considerably increased in substrates that had been incubated with adult flies. The larvae experienced competition at increasing larval densities, expressed by a reduced survival (fig. 4a, statistical trend, $p=0.055$), longer developmental times of the flies (fig. 4b, $p<0.01$) and a reduced thorax length in emerging females (fig. 4c, $p<0.05$). Male thorax length was not significantly affected by larval density (fig. 4d, $p>0.0.5$).

The development of fungal growth on apple chunks (fig. 5) was reduced after incubation with adult flies ($p<0.05$, from third day onwards) and with increasing larval densities (first order terms, $p<0.001$ from the fifth day onwards). On top of the effects of larval and adult density, a high degree of fungal cover correlated with longer developmental times ($p<0.05$) and smaller thorax length for both males and females ($p<0.001$).

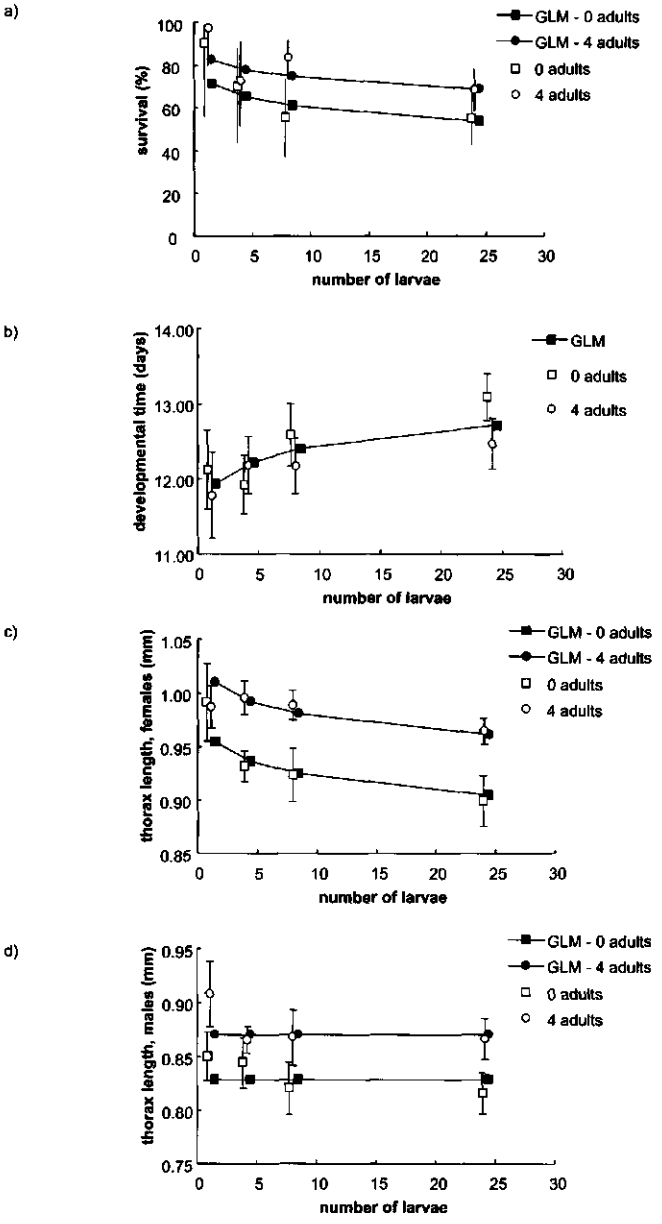

Figure 4: Average fitness components for larvae, reared at different densities in apple chunks, which had been incubated with 0 or 4 adult flies, prior to the introduction of the larvae (see methods). **a)** The survival (+ 95 % confidence intervals) and **b)** developmental time (± s.e.m.) of *D. melanogaster* larvae and the thorax length (± sem.) of emerging **c)** females and **d)** males at different larval densities. The open symbols depict the averages from our data; the closed symbols depict the minimal adequate model, as calculated with Generalized Linear Models (GLM). For clarity, the different series are slightly shifted around the value of x (larval density).

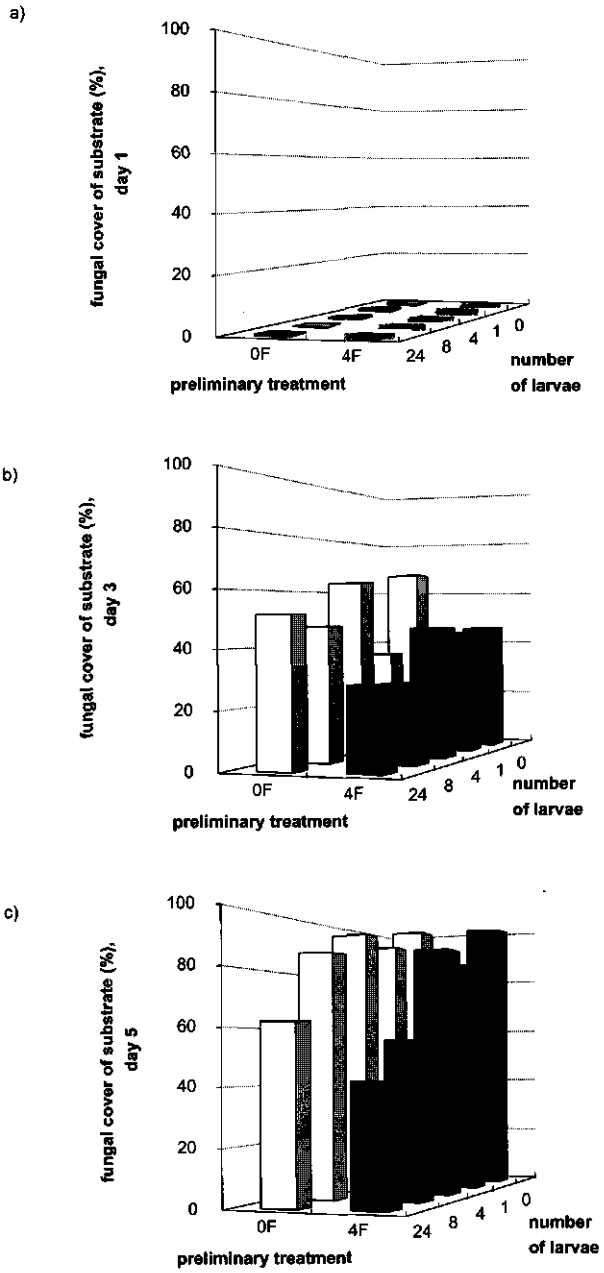

Figure 5: Fungal growth on apple chunks with different preliminary treatments and different numbers of larvae. The average percentages of substrate that was covered with fungi were calculated on angular transformed data and back-transformed.

Discussion

Resource exploitation by increasing densities of *D. melanogaster* larvae in itself did not indicate a component Allee effect, but competition instead, just as was found for *D. subobscura* (Hoffmeister and Rohlfs 2001). Increased larval densities reduced survival, increased developmental time and yielded smaller emerging flies. However, we did find an Allee effect for adult density. Increased density of adult flies on substrates, prior to the introduction of larvae, enhanced larval survival and, in apple chunks, yielded larger flies. For *Drosophila*, larger body size confers a higher fitness (Partridge et al. 1986, McCabe and Partridge 1997, Reeve et al. 2000). Thus, through the effects of adult density on larval fitness components, we revealed significant benefits for the aggregated oviposition in adult female fruit flies. Whether these benefits are sufficient to attribute the evolution of aggregation pheromone in *D. melanogaster* to this component Allee effect will be discussed in relation to the interactions with fungi (see below). The development of fungal growth on the substrates statistically contributed to the observed patterns in larval survival and sizes of emerging flies. A high degree of fungal cover was related to reduced survival in mashed apple and prolonged developmental times and reduced sizes of the emerging flies in the apple chunks. The prior presence of adults on a substrate reduced the fungal growth. Fungal development was also strongly reduced at high larval densities.

It is unclear whether, and, if so, how the fungal growth on substrates affected larval growth and development. Was the reduction in larval survival caused by the fungi, or alternatively, did the fungi prosper because the larvae had died? Literature indicates that fungi can have deleterious effect on *Drosophila* larvae (Atkinson 1979, Ashburner 1989, Hodge et al. 1999), and the diminishing size and increased developmental time of the survivors for increasing degrees of fungal cover in apple chunks suggest that the fungi were in some way harmful to the larvae. If we assume that they were, then did they exert a direct detrimental influence on the larvae through for example toxins, or indirectly, through an interaction with the growth of yeast on which the larvae feed? Similarly, the reduction in fungal growth by prior adult presence on a substrate might have been effectuated directly by inoculation of an anti-fungal agent, or indirectly, through inoculation of yeasts. Fungi and yeast compete for resources in fruit, and any process that favours one of the competitors could result in a shift in the competitive interaction. The tunnelling activities of larvae might destroy fungal mycelium, while the inoculation of substrates with yeast by adults might give the yeast a head start. Some evidence suggests that a mutualistic interaction between *Drosophila* and yeasts exists, that might eventually suppress the development of fungal growth. Firstly, yeast development is faster within insect-infested resources (Fogleman and Foster 1989), and the succession in yeast communities is driven through inoculations by adult *Drosophila* (Morais et al. 1995). Secondly, when baker's yeast was added to standard substrates of mashed apple, no fungal growth was visible (B. Wertheim, unpublished results). Thirdly, female fruit flies were reported to vector a higher diversity of yeasts than males (Morais et al. 1995), which corresponds with our observations of a stronger effect on fungal growth of prior incubation with females than with males. It is therefore likely that the positive effect of adult density on larval fitness partly arose from the inoculation of yeasts, which also

reduced fungal growth. However, the effects of fungal cover and adult density were not fully exchangeable in the statistical models, indicating that other processes also operated.

When we accept that fungi are indeed detrimental for larval development, directly or indirectly, then our results support the hypothesis that a benefit of aggregated oviposition can arise through suppressing fungal growth. This component Allee effect arises at increased adult densities. Whether the aggregation of ovipositing females enhances the resource quality sufficiently to outweigh the effects of increased larval competition, will in general depend on the characteristics of the resource and the residing micro-biota. It is noteworthy that the beneficial effects of prior presence of adults were clearly more pronounced in the apple chunks (a demanding substrate for the larvae) than in the mashed apple substrates (a fairly benign substrate). Furthermore, the marked decrease in fungal development at high larval density might result in additional benefits, when the fungal species are more noxious than those that infested the substrates in these laboratory experiments.

To translate our results on component Allee effects to the scale of total fitness, i.e. to a demographic Allee effect, would require many additional experiments, for example on other fitness components (an inadequate yeast diet can cause reduced and delayed fertility and fecundity (Dudgeon 1954, Cooper 1959) and in other substrate types (Courtney et al. 1990). Furthermore, stochasticity in mortality risk should explicitly be incorporated in an analysis for a demographic Allee effect, since aggregation might reduce mortality only under adverse conditions, whilst the advantage is lost under ideal conditions (Lockwood and Story 1986). Moreover, all ecological interactions that are affected by the use of aggregation pheromone must be incorporated in a cost-benefit analysis, to judge when the use of aggregation pheromone would be promoted by natural selection.

In the introduction we stated that aggregative behaviour can evolve, provided that already at low densities benefits outweigh all costs. The mechanism that was investigated in this study could produce such a scenario when the component Allee effects result in a demographic Allee effect. When on average the fitness of an ovipositing female, comprising the quantity and quality of her offspring, is enhanced when another female shares the same oviposition substrate, both females benefit when seeking out each other. The aggregation pheromone in *Drosophila* is male-produced, and transferred to females during copulation, together with many other male accessory gland products (Bartelt et al. 1985b, Partridge et al. 1986, Chapman et al. 1995). Therefore, the physiological costs of producing the pheromone are none for the female. Recently mated females have both a high emission rate of pheromone and a high oviposition rate (Bartelt et al. 1985b, Partridge et al. 1986), making the pheromone a reliable indicator for the presence of other gravid females. Finally, aggregating insects can be more successful in transmitting micro-organisms and causing rot than individuals alone (Fleischer et al. 1999). Since rot and fermentation are exactly what renders a substrate suitable for *Drosophila* larval development (Atkinson and Shorrocks 1977), this is in strong agreement with our suggestion that aggregation pheromone has evolved in response to a benefit of aggregated oviposition. Clearly, the topic deserves much more study to clarify all 'ifs' and 'whens'. Such an effort may also shed light on the numerous other insect species that both possess aggregation pheromone and have a mutualistic interaction with micro-organisms (chapter 2). Possibly, the evolution of aggregation pheromone is somehow facilitated or even directed by the interaction with the micro-organisms.

Acknowledgements

We gratefully acknowledge Dine Volker for identifying the fungi, Frodo Kindt for lending the eyepiece micrometer, and Leo Koopman for culturing the flies.

CHAPTER 6

Increased risk of parasitism as ecological costs of using aggregation pheromone: Laboratory and field study of *Drosophila* – *Leptopilina* interaction

Abstract

Information conveyance plays an important role in parasitoid–host interactions. Several sources of information are available for searching parasitoids and exploitation of that information during the different phases of host location depends on its reliability, detectability and accuracy. One source of information especially suitable for exploitation by parasitoids is host aggregation pheromone, because this often combines all three aspects. In laboratory and field experiments we studied the behavioural responses of the parasitoid *Leptopilina heterotoma* to the aggregation pheromone of the fruit fly *Drosophila melanogaster*, both for substrate selection and the behaviour on substrates. Our results show an increasing numerical response towards substrates with increasing dose of the host's aggregation pheromone, whereas we found no significant effects of pheromone on searching behaviour on substrates. Searching behaviour on substrates was influenced by other host cues, which is in accordance with the expectations from reliability–detectability criterions. The responses of the parasitoids were further influenced by substrate quality (i.e. yeast concentration) and the microscale distribution of pheromone. In several field experiments, the fraction of fruit fly larvae that was parasitized was significantly higher in substrates with aggregation pheromone than in control substrates, indicating an ecological cost to the use of aggregation pheromones in adult *D. melanogaster*.

Introduction

Many behaviours of animals convey information on their whereabouts, their identity and their activities. Such information plays an important role in the interaction with their natural enemies. Depending on the source of information, and on the cost and benefits to all participants, selective forces shape the information conveyance (Dicke and Vet 1999, Dicke and van Loon 2000). Information conveyance is also important in the interaction between insect parasitoids and their hosts (Godfray 1994). Parasitoids can exploit inevitable cues that originate directly from the host itself, e.g. frass, movement vibrations or feeding traces (Casas 1989, Meyhöfer et al. 1994, Mattiacci et al. 1999). In these situations, directional selection acts on the host to minimize the information conveyance, to become as inconspicuous as possible. A more complex situation arises when the parasitoid is eavesdropping on deliberate cues for intraspecific communication between the hosts, because those often comprise a function to the hosts, for example volatile pheromones, marking trails or mating calls, (Dicke and Sabelis 1992, Gray and Cade 1999, Hoffmeister et al. 2000). This becomes even more intricate, when the communication occurs in another host stage than the one under parasitoid attack, such as with egg- or larval parasitoids, eavesdropping on the communication between adult hosts (the 'infochemical detour', Vet et al. 1991). In such situations, opposing selection forces act on the intraspecific communication among hosts, shifting between divergent costs, benefits and constraints for each participant. Especially when the intraspecific communication is vital for the hosts, a parasitoid has a magnificent opportunity for an exploitative strategy, while the host is stuck in an unfortunate situation: One woman's meat is another woman's poison.

Firstly, we focus on the hosts' perspective. When intraspecific information conveyance benefits both the emitter and the receiver, as for example in mate finding, directional selection drives both receivers and emitters to an evolutionary state where communication is necessary. When a (severely) detrimental side-effect of communication arises, such as espionage by a parasitoid, it is no longer possible to simply stop communicating. The 'fitness trough' that phenotypic evolution has to go through before reaching another 'fitness peak' can impede the loss of such a fundamental trait. In these situations, selection can at most slightly alter the trait, for example by restricting signalling to a particular time window. How heavy the costs of intraspecific communication then get, depends very much on the behaviour of the spying parasitoid.

Now we will focus on the perspective of the parasitoid. The behaviour of searching parasitoids is optimised by natural selection to deal with the tremendous difficulty of finding tiny hiding hosts in a huge heterogeneous world (van Alphen and Vet 1986, Vet et al. 1991, Godfray 1994). Most parasitoids are expected to be time limited, meaning that they die before they have depleted their eggs (Sevenster et al. 1998, Ellers et al. 2000). Time limitation selects for increased rate of offspring production, or increased efficiency. Parasitoid use cues that guide them to the (few) suitable habitats that are occupied by hosts (Vet and Dicke 1992), and thus experience enhanced efficiency (Papaj 1993). There is, however, a major problem with using directional cues: The most reliable cues are the least detectable ones, because hosts are under selection to be as inconspicuous as possible (Vet et al. 1991, Vet and Dicke 1992). Reliability

comprises information on the presence of potential hosts, their species, age, and numbers. The more accurate the cues are, the more efficient the parasitoid can operate.

During the full chain of processes leading to final host location, the parasitoid will have to switch constantly between the available stimuli to use those that are detectable and the most accurate. Parasitoids have evolved strategies to deal with the reliability-detectability problem and they combine pieces of information during all phases of host searching. This can, for example, involve information from a third party, such as with the infochemical detour (Dicke and Sabelis 1992, Vet and Dicke 1992, Stowe et al. 1995, Haynes and Yeargan 1999), with plants that produce volatiles in response to herbivory (Vet and Dicke 1992, Stowe et al. 1995) and with associatively learned non-host cues (Vet and Schoonman 1988, Papaj and Vet 1990, Vet et al. 1998).

In this paper, we study the ecological costs of communication for hosts, that arise from an eavesdropping parasitoid. How important are the pheromones in the dynamic process of host location? Our study system comprises the fruit fly *Drosophila melanogaster* Meigen and the larval solitary parasitoid *Leptopilina heterotoma* (Thomson). Laboratory studies showed that this parasitoid can perceive the aggregation pheromones of adult fruit flies and thus, she may use that information to locate her larval hosts (Wiskerke et al. 1993a, Hedlund et al. 1996a). The major active compound of *D. melanogaster*, cis-vaccenyl acetate, is produced by males, and transferred to females during copulation (Butterworth 1969, Brieger and Butterworth 1970, Bartelt et al. 1985b). Especially recently mated female fruit flies emit the aggregation pheromone while on substrates, which attracts other males and females and induces aggregated oviposition (Bartelt et al. 1985b, chapter 3 and 4). The most likely intraspecific benefit of using aggregation pheromone in *D. melanogaster* is aggregating the offspring on fermenting substrates for communal resource exploitation (chapter 5). Thus, the adult pheromone is linked to the larval survival probabilities and therefore vital and indispensable. To quantify the ecological costs of pheromonal communication with respect to risk of parasitism, the behavioural responses of the parasitoid should be known.

The parasitoid *L. heterotoma* has a variety of cues at her disposal, differing in reliability (especially accuracy) and detectability. She can quantitatively estimate larval numbers after arrival on a substrate through larval contact kairomones (Dicke et al. 1985), but she is unable to perceive these non-volatile cues from a distance (Dicke et al. 1984) (i.e., high reliability and accuracy, but low detectability). She can detect volatile substrate odours from a distance and use these for habitat selection, and her behavioural responses strongly increase after she experienced the cues to be profitable (Vet 1988, Papaj and Vet 1990, Vet et al. 1998) (i.e., high detectability, moderate reliability but low accuracy). She can also detect the aggregation pheromone of adult fruit flies in the laboratory and may use that in substrate selection in the field (Wiskerke et al. 1993a, Hedlund et al. 1996a). The aggregation pheromone is a relatively reliable cue for *Leptopilina* because (1) it is mainly emitted by recently mated fruit fly females, that have high oviposition rates (Herndon and Wolfner 1995) and (2) it is persistent for several days, bridging the time-gap between the oviposition by fruit flies and the larval stage that is suitable for the parasitoid (Wiskerke et al. 1993b). Additionally, the accuracy is potentially high, because aggregation pheromones in *Drosophila* are fairly species-specific and *Leptopilina* spp. indeed respond to differences in pheromones of several *Drosophila* species (Hedlund et al. 1996a). Furthermore, aggregation pheromone give

quantitative information, because fruit fly numerical responses increased with increasing pheromone dose in a field study (chapter 3).

To study the ecological costs of communication for *D. melanogaster* with respect to increased risk of parasitism, we address the following question: Which aspects of host searching behaviour of *L. heterotoma* are affected by the presence of aggregation pheromone of *D. melanogaster*? In a windtunnel, we studied the parasitoid's chemotactic response during substrate selection, in relation to other substrate characteristics. In behavioural observations, we studied the searching behaviour after arrival on the substrate, in particular arrestment responses and time allocation. To quantify ecological costs, it is essential to do tests under natural conditions, where the full array of stimuli is available (Casas 2000). We therefore conducted additional experiments in a semi-field set-up and in the field. We tested the combined behavioural responses of the parasitoid to fruit fly aggregation pheromone and calculated the risk of parasitism for *D. melanogaster* larvae in the presence and absence of aggregation pheromone.

Material and methods

Study organisms

Cultures of *Drosophila melanogaster* and *Leptopilina heterotoma* were started in 1995 and in 1998 with individuals that were reared from apples, collected in an orchard in Wageningen, the Netherlands. The insects were reared at 20 ± 1 ° C and 16:8 L:D on artificial substrates: *D. melanogaster* on a medium of dried bakers' yeast, sugar and agar (40 g, 135 g resp 23 g in 1 l of water); *L. heterotoma* on *D. melanogaster* larvae feeding on a medium of 'mashed apple' (Golden Delicious, mashed in a blender with approximately 150 ml water added to each litre of pulp, frozen upon preparation and defrosted before use), dried bakers yeast, sugar and agar (500 g, 40 g, 20 g resp 20 g in 1 l of water). Populations of mature fruit flies were kept in cages under the same conditions. Mature wasps were kept in vials with water agar (20 g agar in 1 l of water) at 12.5 ° C and 16:8 L:D. In the experiments, we used naive female parasitoids, 8-12 days after emergence.

To obtain *D. melanogaster* larvae for experiments, vials with water agar and a thin layer of living bakers yeast (Fermipan, Gistbrocades, 250 mg / ml water) were offered to a population of *D. melanogaster* for oviposition, and larvae were rinsed from this medium after reaching the second instar. Larvae were thoroughly rinsed with water to remove the coating of yeast before adding them to experimental test substrates.

Pheromone

The major active compound of the aggregation pheromone of *D. melanogaster* is (Z)-11-octadecenyl acetate, common name cis-vaccenyl acetate (Bartelt et al. 1985b). For experiment, the synthetic compound (99% pure, Pherobank, Wageningen) was diluted in hexane (this dilution is from hereon referred to as 'cVA'), and applied in a standard dose of 4.5 μg in 15 μl hexane, which is approximately the

equivalent of the deposition by 15 recently mated female *D. melanogaster* (Bartelt et al. 1985b). The control treatment for synthetic pheromone application consisted of 15 µl hexane ('hexane').

Alternatively, pheromone application was acquired through natural deposition by *D. melanogaster*. Either 12 virgin females plus 12 virgin males ('FM') or 24 recently mated females (immediately after breaking copula: 'F*') were incubated on 4 substrate patches, in a closed container (12 x 25 x 10 cm) at 20 °C, for 16-18 hours (laboratory experiment) or 6 hours (field experiments). The control for this treatment was incubation of 24 virgin females ('F') on 4 substrate patches. Prior to the experiments with naturally deposited pheromone, all eggs were removed from the patches with a pair of tweezers and patches were smoothened with a spatula; the surfaces of the control patches were also disrupted with a separate pair of tweezers and subsequently smoothened with another spatula. The numbers of eggs and faecal droppings were recorded as a measure of fly activity. Virgin flies were obtained by separating male and female pupae, 1-2 days prior to emergence (male sex combes on the front tarsi are visible through the pupal case).

Standard substrates

For all experiments, the aggregation pheromone of *D. melanogaster* was applied on top of a standard substrate. These substrates were prepared of mashed apple ('A'), mashed apple mixed with living bakers yeast in a specified w/w ratio (from hereon 'apple-yeast mixture' or 'AY'), or living bakers yeast ('Y', 1 ml suspension [250 mg / ml water]). The substrates were offered as 'patches' (2.5 cm ⌀, 0.5 cm height), in petri dishes or the lid of 175 ml Greiner containers (both referred to as 'petri dish': 8 ml of substrate, 5 cm ⌀) or in 'discs' (14 cm ⌀, 1 cm height, PVC) with 50 micropatches (holes of 12 mm ⌀, 4 mm depth, each filled to the rim with substrate). Standard substrates were either pheromone-treated (synthetic pheromone or through natural deposition), or controls (hexane or virgin females). Patches and petri dishes with synthetic pheromone received one standard application of cVA or hexane. For the pheromone-treated discs, topical applications of cVA (standard doses) were added to 5 of the micropatches per disc; in the control discs 15 µl hexane was added to 5 of the micro patches. The discs allowed for observing the behavioural effect of pheromones on both a macroscale (whole disc, symbolising a fruit item) and a microscale (hole specific, symbolising the heterogeneity within each fruit item). Petri dishes were used when larvae were added to the substrates to investigate rates of parasitism. After experimentation, the petri dishes were covered with Greiner containers (175 ml), in which a hole was punctured and plugged with cotton wool for ventilation. The containers were incubated in a shelter (15-25 °C) to rear all insects until adulthood. Table 1 summarizes the types of substrates that were used for each experiment.

Windtunnel experiments

The chemotactic response of parasitoids towards substrates with the aggregation pheromones of *D. melanogaster* was tested in a windtunnel set-up. The windtunnel (40 x 50 x 40) consisted of 2 solid sides, a glass floor and ceiling (30 x 40cm), resting on a 10 cm wide rim. The front was open and the back was covered with gauze (mesh width 1 mm). The windtunnel was placed in a climatized room, at 22 ± 1 °C and 70 % RH. To obtain an airstream, we used the air circulation of the climatized room, which is

Table 1: Summary of the experiments in each set-up. Standard substrates either consisted of an apple-yeast mixture (AY) of specified w/w ratio, mashed apple (A) or yeast (Y). They were offered as patches, in petri dishes or in discs (see methods). Test substrates were either treated with aggregation pheromone (i.e., synthetic: cVA; recently mated females: F* or females plus males: FM) or a control treatment without pheromone (hexane, virgin females: F, no flies: 0), and the behavioural responses of parasitoids were compared for these treatments. Larvae were added to compare parasitism in the presence and absence of aggregation pheromone. See 'methods' for further explanation and used abbreviations.

experiments	pheromone treatment	larvae added	test substrate
windtunnel			
1) synthetic pheromone	cVA - hexane	-	patch: AY (15:1)
2) naturally deposited pheromone	F* - F	-	patch: A
3) synthetic pheromone, substrate quality (yeast concentrations)	cVA - hexane	-	patch: sterilized A, A, A1d, AY (150:1), (75:1), (15:1),(15:1)1d
on-patch behaviour			
1) synthetic pheromone	cVA - hexane	-	patch: AY (15:1)
2) naturally deposited pheromone	F* - F - 0	-	patch: A
3) synthetic pheromone	cVA - hexane	2, 8	patch: Y
population cage			
1) synthetic pheromone	cVA - hexane	-	disc: AY (50:1)
2) naturally deposited pheromone, larvae added	FM - F	8	petri dish: AY (50:1)
field			
1) synthetic pheromone	cVA - hexane	-	disc: AY (100:1)
2) synthetic pheromone	cVA - hexane	16	petri dish: AY (100:1)
3) naturally deposited pheromone	FM - F (24, 50-200 flies)	16	petri dish: AY (100:1)
4) synthetic pheromone, fly traces on all substrates	all F: cVA - hexane	16	petri dish: AY (100:1)
5) dose response	cVA (0.45 μg - 4.5 μg - 45 μg) - hexane	16	petri dish: AY (100:1)
6) various numbers of larvae, cVA added to all substrates	all cVA	4-8-16-32	petri dish: AY (100:1)
7) control experiment, synthetic pheromone, renewing substrates	cVA - hexane, substrates renewed every 40 min.	16	petri dish: (AY 100:1)
8) superparasitism,	cVA - hexane	16 (dissected)	petri dish: (AY 100:1)
9) parasitism after short exposure	cVA - hexane (3 hours)	16	petri dish: (AY 100:1)

continuously filtered over active charcoal and subsequently pressed through holes in one wall (⌀ 1 mm, approximately 2 cm apart), thus creating a fairly but not too laminar airflow. The distance between the back of the windtunnel and the wall was adjusted such that the wind speed at the release point in the windtunnel was 0.3-0.4 m/s. Prior to each windtunnel test, a 'control test' was run with a set of two substrate patches that were known to be non-attractive, to ensure that the environment was not contaminated with pheromones from previous windtunnel experiments (Howse et al. 1998). When responsiveness in the control test series was above 30 % (10 females tested), experiments were abandoned. The floor glass plate was regularly cleaned with ethanol.

Two test substrates were placed 10 cm apart on the floor of a windtunnel, one treated with pheromone and the other with the control treatment. Female parasitoids were isolated in glass vials (⌀ 1.0 cm, length 5cm), and a single female parasitoid was released 25 cm downwind of the two test substrates by placing the opened vial horizontally on the floor of the windtunnel. Females made a choice by walking towards one of the substrate patches, often in a straight line, and just before the females reached a substrate patch they were removed from the set-up. Choice (control or treatment patch) and responsiveness (choice or no choice) were noted. A female was scored as unresponsive when she had not arrived at one of the substrates within 5 minutes after release or when she flew off. For each experiment, the response and choice of 30 – 50 female parasitoids was tested. Each series of 10 females was tested with a new set of patches. Positions of treated and control substrate patches were regularly changed during each series.

We compared the attractiveness of substrates with (1) synthetic pheromone (cVA) and hexane; (2) naturally deposited pheromone (recently mated females F*) and virgin females (F, no pheromone deposition); (3) synthetic pheromone (cVA) and hexane on different substrates, to test the effect of substrate quality (i.e. yeast concentration) on the chemotactic response to the pheromone (table 1). The range of different yeast concentrations for (3) was obtained by using (a) heat-sterilized mashed apple, (b) mashed apple 'A', (c) mashed apple, left at room temperature for 24 hours 'A, 1 day', (d) poor apple-yeast mixture (AY 150:1 w/w), (e) intermediate apple-yeast mixture (AY 75:1 w/w), (f) rich apple-yeast mixture (AY 15:1 w/w), (g) rich apple-yeast mixture left at room temperature for 24 hours (AY 15:1 w/w, 1 day).

On-patch behavioural experiments

To test whether the behaviour of searching parasitoids on substrates is affected by the presence of aggregation pheromones of *Drosophila*, behavioural observations were conducted on individual *L. heterotoma* females, searching on artificial substrate patches (as described below). The experiments were conducted in a climatized room (20 ± 3 ° C) under a stereo microscope in an airtight container (11 cm ⌀, 5 cm height for experiment (1 and 2) and 5.0 cm ⌀, 2.0 cm height for experiment (3)). In the large container (experiment 1 and 2), a small airstream was created and the incoming air was filtered over active charcoal and hydrated over tap water. The behaviour of individual female wasps was observed on a substrate patch (each replicate patch with a new wasp). A female that would not enter the patch, or left the patch within 30 seconds was replaced by a new female on the same patch upon a maximum of 6 trials. After six failures, the replicate patch was discarded and not included in the statistical analyses.

The female wasp was gently introduced by letting her step onto the patch and her behaviour was recorded for as long as she chose to remain on the patch. Leaving a patch was defined as flying off, or as walking off to a distance greater than 1 cm and not returning within 1 minute. The behaviours that were distinguished were walking, probing with the ovipositor, ovipositing, standing motionless, standing still with alternating antennal movements, preening and wing vibrations. The beginning and end of each behaviour was recorded with the software package The Observer (Noldus Information Technology, Wageningen, the Netherlands) on a handheld computer (Psion Workabout).

The behaviours on the following sets of treatments were compared (see table 1): Standard substrate patches with (1) synthetic pheromone (cVA) and hexane (30 replicates per treatment);(2) naturally deposited pheromone (recently mated F*), virgin females (F, no pheromone deposition) and no flies (0) (30 replicates per treatment); (3) synthetic pheromone (cVA) and hexane with 2 or 8 second instar *D. melanogaster* larvae (15 replicates per treatment); larvae were added half an hour before experimentation. After the behavioural observations in experiment (3), the larvae were dissected to determine the rate of parasitism.

Outdoor population cage experiments

To study the effects of fruit fly aggregation pheromone on parasitoid searching behaviour and rate of parasitism in a more natural and complex environment, parasitoid responses were tested in a large outdoor population cage. The population cage (3 x 6 x 2 m) consisted of 4 walls and a ceiling of fine gauze, tied underneath two adjacent party tents (3 x 3 x 2 m each). This construction was put up on a stretch of grassland in the open air. The gauze sidewalls were secured on the grassland with bricks.

For each experiment, 4 control substrates and 4 pheromone-treated substrates were placed on the ground in the population cage in 2 rows (1 m apart), treatment and control substrates alternating (1 m apart). After placing the substrates on the grass, 400 - 800 *L. heterotoma* (sex ratio 1:1) were released by placing their holding pots horizontally on the floor in between the two rows, 1 m from the first two substrates, and removing the plug. Typically, the parasitoids would immediately climb to the highest point of the pots and take off. From the moment of release, the numbers of males and females on each test substrate were counted every 5 minutes for 3 hours. These counts combine chemotactic and arrestment responses.

We compared the response of *L. heterotoma* to substrates with (1) synthetic pheromones (cVA) and hexane; (2) naturally deposited pheromone (FM, virgin females plus males) and virgin females (F) (see also table 1). For experiment (2), 8 second instar *D. melanogaster* larvae were added to the substrates half an hour before the experiments started. The rate of parasitism was determined by rearing the insects from the substrates in a climate room (20 °C, 16:8 L:D). Each experiment was repeated on 3 days.

Field experiments

To evaluate the effects of *D. melanogaster* aggregation pheromones on the searching behaviour of *L. heterotoma* in a natural environment, where the full array of stimuli is available for host location, we conducted field experiments in a low maintenance orchard in southern France (ecological parcel of the INRA field station, Gotheron (Drôme)), from mid-August until mid-October 1999. We offered pheromone-treated standard substrates and control substrates to the naturally occurring parasitoid population, comprising mainly *L. heterotoma* and *L. boulardi* (emergence ratio from natural substrates 1:3, chapter 3). *Leptopilina boulardi* also responds to the aggregation pheromone of *D. melanogaster* in windtunnels and olfactometers (Hedlund et al. 1996a, Couty et al. 1999). The most common *Drosophila* species in the orchard were *D. melanogaster* Meigen 1830, *D. simulans* Sturtevant 1921, *D. hydei* Sturtevant 1921, *D. immigrans* Sturtevant 1921 and *D. subobscura* Collin 1936 (chapter 3).

Similar to the population cage experiments, sets of 4 control substrates and 4 pheromone-treated substrates were placed on the ground in 2 rows in alternating order (2-3 m between rows and 2 m between patches in each row), between the fallen apples (0-25 apples / m^2). The numbers of parasitoids on each patch were counted every 10 minutes for 3 hours (weather and light conditions permitting). Experiments started at 5 pm, because pilot experiments showed the highest activity level for parasitoids in the hours around dusk (see also Fleury et al. 2000). After the three hours of observation, the substrates were left in the field for parasitization until the next morning. The insects were reared until adulthood to determine the percentage of hosts parasitised. Each set of treatments was tested on 3 different days.

Except for the first experiment, standard substrates were offered in petri dishes and infested with 16 second instar *D. melanogaster* larvae half an hour before the experiment. We tested standard substrates: (1) with standard applications of cVA or hexane (discs, no hosts); (2) with a standard application of cVA or hexane; (3) with naturally deposited pheromone by 12 female plus 12 male flies (FM) or the control 24 virgin females (F), also tested for higher densities of flies (50-200); (4) all substrates first incubated with 24 virgin flies (F), and then with an application of cVA or hexane; (5) with an application of three different doses of cVA (0.45 μg, 4.5 μg or 45 μg in 15μl hexane) or hexane; (6) all with a standard dose of cVA and 4, 8, 16 or 32 second instar *D. melanogaster* larvae added (table 1). In set (5) and set (6), for each of the four treatments, four patches were offered simultaneously, thus resulting in a set of 16 substrate patches, instead of the regular 8. For experiment (6), no counts were made on the numbers of parasitoids on the patches, but percentage parasitism was recorded exclusively. A control experiment (7) was performed to ensure that attractiveness of the patches was not influenced to a large degree by fruit flies that visited the patches during the experiments, and that may have deposited pheromone. Four sets of type (2) were prepared instead of the regular one set. The patches were used for only 40 minutes and then replaced by a new set in a different locality of the orchard. In this way we reduced the impact that the flies might have had on the patches. To get an impression of the rate of super- or multiparasitism (8), a set of substrate patches type (2) was placed in the field as for the other tests, and larvae were dissected to count the number of parasitoid eggs. To evaluate rates of parasitism during the 3 hour observational period (9), an additional set of type (2) was collected from the field directly after the behavioural assay.

Statistical analyses

In the windtunnel experiments, the numbers of females that chose for the control and for the treatment patches were compared with a G-test (extrinsic null hypothesis of equal attractiveness), with Williams correction for a better approximation of the chi-square distribution (Sokal and Rohlf 1995).

For the behavioural observations on substrates, we calculated the total duration of each behaviour, the mean bout duration of each behaviour and the total time spent on the patch (the Observer Software, Noldus Information Technology). The behaviours 'total probing time' (= total duration + 0.2 to avoid exclusion of zeros), 'mean bout length probing' (only for those parasitoids that did probe) and the 'residence time' were compared between treatments with a Generalized Linear Model (GLM) with gamma distribution and Log link function (SAS, v. 6.12: Proc Genmod, the scale parameter was estimated by dividing deviance by the degrees of freedom (option dscale)) and subsequent Wald Tests with a Bonferroni correction. The numbers of eggs and faecal droppings were included as covariables for the analysis of

naturally deposited pheromones. The numbers of failures were compared with a GLM with Poisson distribution and Log link function; the fractions of parasitised larvae and the percentage of females that did probe on the patches was compared with GLMs with Binomial distribution and Logit link function.

For the population cage and field experiments, the cumulative numbers of parasitoids (summed over the full observational period) on the pheromone-treated and control patches were compared with a GLM with Poisson distribution and Log link function (standard errors were adjusted with dscale to correct for overdispersion). The days were included as a block factor, and the cumulative numbers of fruit flies that visited the patches (field experiment) were included as covariables. The estimates of the parameter values of the GLM were back transformed to obtain a 'Factor of Difference' , which describes the ratio in parasitoid numbers on pheromone-treated substrates and control substrates ($N_{pheromone}/N_{control}$), that can be attributed to the presence of pheromone. The fractions of larvae that were parasitized (p) on treated and control substrate were compared with a GLM with Binomial distribution and Logit link function. For these analyses, the 'Odds Ratio' for pheromone treatment is calculated, describing $(p_{pheromone}/(1-p_{pheromone})) / (p_{control}/(1-p_{control}))$. In the population cage, the response of females to the microscale distribution of pheromone was tested by comparing the fraction of female counts on the treated micropatches between cVA discs and hexane discs. For the different doses of cVA, the dose was log-transformed (Ln(dose + 1)) and tested as a linear and quadratic continuous predictor.

The identically treated substrates for each experiment were considered as independent replicates, although within days they were drawn simultaneously from the same local population, and could therefore be considered 'pseudo replications' (Hurlbert 1984). However, the parasitoid populations in the field and the population cages were so large, that the chances of one substrate interfering with another substrate were considered to be sufficiently small to treat them as independently sampled.

Results

Windtunnel experiment

Leptopilina heterotoma chose significantly more often for odours from substrates with pheromone than those from control substrates (fig. 1a), both when tested with synthetic pheromone (cVA versus hexane: $G_{adj} = 5.51, p < 0.05$) and with naturally deposited pheromone (F* versus F: $G_{adj} = 9.33, p < 0.01$). The percentages of parasitoids responding in the windtunnel experiments were 72 % with synthetic pheromone and 88 % with naturally deposited pheromone. For the control (no flies (0) versus no flies(0)), only 6 % of the parasitoids was attracted to the substrates.

The concentration of yeast in the artificial substrate patches strongly affected the chemotactic behaviour of *L. heterotoma*, and significant attraction towards the aggregation pheromone of *D. melanogaster* was found only for substrates with relatively high concentrations of yeast (fig. 1b, AY (75:1):$G_{adj} = 6.15, p < 0.05$; AY(15:1): $G_{adj} = 6.72, p < 0.01$). With increasing concentration of yeast, we initially observed an increase in responsiveness, then a significant higher number of choices for substrates with pheromone, and finally a slight decrease in this preference (fig. 1b).

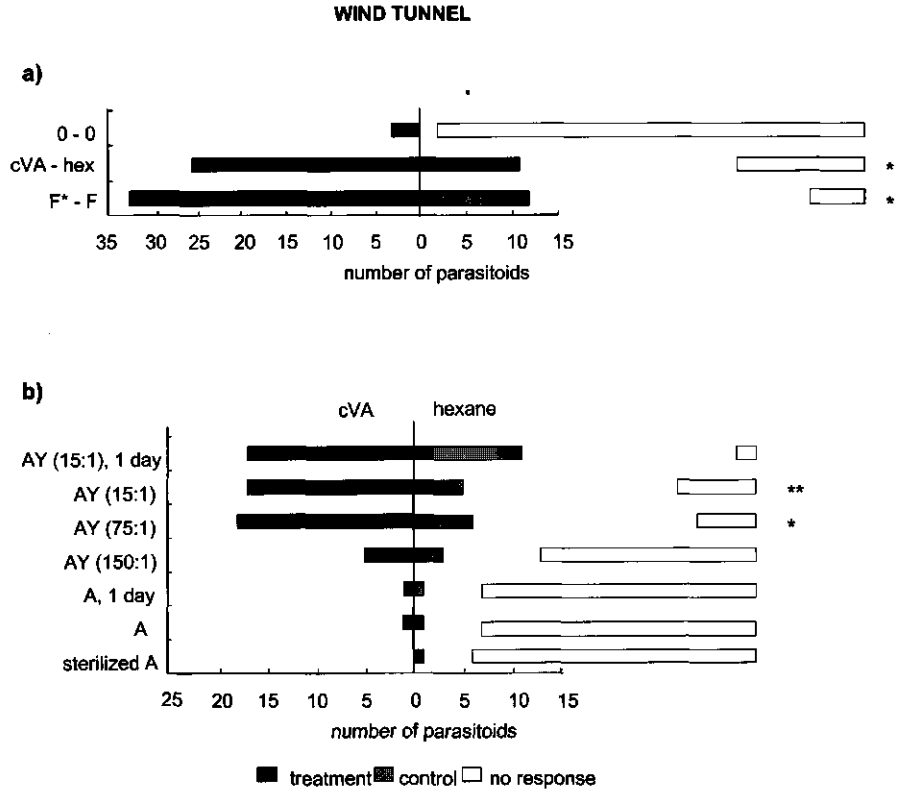

Figure 1: Numbers of female *L. heterotoma*, choosing for pheromone-treated and control substrates in a windtunnel setup. **a)** Chemotactic response to standard substrates (see also table 1) without pheromone or fly traces (0), with synthetic pheromone (cVA) *vs* the control hexane (hex), and with naturally deposited pheromone (recently mated females, F*) *vs* the control virgin females (F). **b)** Effect of substrate quality on the chemotactic response to synthetic pheromone. Chemotactic responses to synthetic pheromone (cVA) *vs* the control hexane on substrates, consisting of mashed apple (A) or apple-yeast mixture (AY) with varying yeast concentrations (w/w ratios). Substrates were used immediately after preparation or were kept at room temperature for 24 hours (1 day).

On-patch behavioural experiments

The on-patch behaviour of *L. heterotoma* was not significantly affected by the presence of aggregation pheromone, but it was affected by other cues (fig. 2, table 2). Traces of adult flies (experiment 2) significantly increased the probing times, probing bout length and residence time of *L. heterotoma*, compared to substrates without any fly traces, but these time-measures were not different for the traces of 'virgin females' F (that do not contain aggregation pheromone) and 'recently mated females' F* (that include aggregation pheromone). An increase in larval density (experiment 3) also resulted in longer total probing time and residence time (table 2). The average probing bout length was shorter at high larval density because of increased host encounter rates, and the percentage of hosts that was parasitized was not significantly different for pheromone treatment or larval density.

Table 2: Time allocation of *L. heterotoma*, searching on pheromone-treated patches and control patches. Total probing time, percentage of wasps that did probe, length of probing bouts (only for those parasitoids that did probe) and total residence times were compared for parasitoids on: (1) Apple-yeast patches with synthetic pheromone (cVA) and the control hexane; (2) mashed apple patches with naturally deposited pheromone (recently mated female fruit flies (F*)), the traces of virgin female fruit flies (F) and patches without fruit fly traces (0); (3) yeast patches with synthetic pheromone and the control hexane with 2 or 8 *D. melanogaster* larvae added. 'Refusals' denotes the numbers of parasitoids that refused the patches; 'Parasitism' is the average percentage of larvae parasitized. Different letters within columns indicate significant differences between rows, as analysed for each of the 3 experiments separately (GLM, subsequent Wald tests and Bonferroni correction). Note that it is not possible to compare the measurements for the three experiments, since the they were not run simultaneously and patches consisted of different materials. Significance is denoted by: ns ($p>0.05$), * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$).

Experiment	probing time (average ± s.e.)	wasps probing	probe bout (average ± s.e.)	residence time (average ± s.e.)	refusals	parasitism
1) synthetic pheromone	ns	ns	ns	ns	ns	
cVA	292.37 ± 42.99	83 %	200.66 ± 38.44	435.19 ± 49.20	22	-
hexane	193.54 ± 37.49	63 %	140.67 ± 33.20	380.64 ± 48.25	24	-
2) naturally deposited	***	***	*	***	**	
mated females (F*)	425.41 ± 45.33 [a]	100 % [a]	175.52 ± 20.56 [a]	561.49 ± 48.66 [a]	10 [a]	-
virgin females (F)	459.12 ± 53.74 [a]	93% [b]	200.84 ± 26.58 [a]	583.75 ± 60.23 [a]	32 [a b]	-
no flies (0)	4.79 ± 2.79 [b]	13 % [c]	36.57 ± 10.58 [b]	166.91 ± 41.39 [b]	50 [b]	-
3) synthetic pheromone with larvae	***	ns	***	***	ns	ns
cVA, 2 larvae	718.31 ± 103.80 [a]	100 %	263.86 ± 27.01 [a]	902.45 ± 139.61 [a]	2	41 %
hexane, 2 larvae	949.20 ± 127.84 [a]	100 %	231.19 ± 27.76 [a]	1163.72 ± 169.61 [a]	4	53 %
cVA, 8 larvae	1201.69 ± 101.30 [b]	100 %	145.37 ± 10.76 [b]	1778.00 ± 187.05 [b]	0	51 %
hexane, 8 larvae	1380.20 ± 145.69 [b]	100 %	135.13 ± 9.16 [b]	1896.56 ± 200.13 [b]	3	64 %

In the experiment with synthetic pheromones (1), no significant differences were found for total probing time, the percentage of females probing, probing bout lengths or residence times (table 2). In the experiments with naturally deposited pheromones (2), the only difference in parasitoid behaviour between the treatments mated (F*) and virgin (F) females was the percentage of females that started probing the patches. Probing times were longer on substrates with larger numbers of faecal droppings (GLM, $p<0.05$), but the numbers of (removed) eggs had no significant effect.

Outdoor population cage experiments

In the outdoor population cage experiments, the cumulative numbers of *L. heterotoma* females were significantly larger on pheromone-treated substrates than on control substrates (table 3, fig. 3), both when tested with synthetic pheromone (GLM: $p<0.001$) and with naturally deposited pheromone (GLM: $p<0.05$). On the cVA and hexane discs with 50 micropatches, we observed a significantly larger fraction of the females on the 5 cVA-treated micropatches than on the 5 hexane-treated micropatches (11 % of counts on cVA micropatches vs 0 % on hexane micropatches, GLM: $p<0.01$), indicating a response of the parasitoids to the microscale distribution of pheromone. The fractions of larvae that were parasitized were not significantly different between pheromone (FM) and control (F) treatments. Mortality during

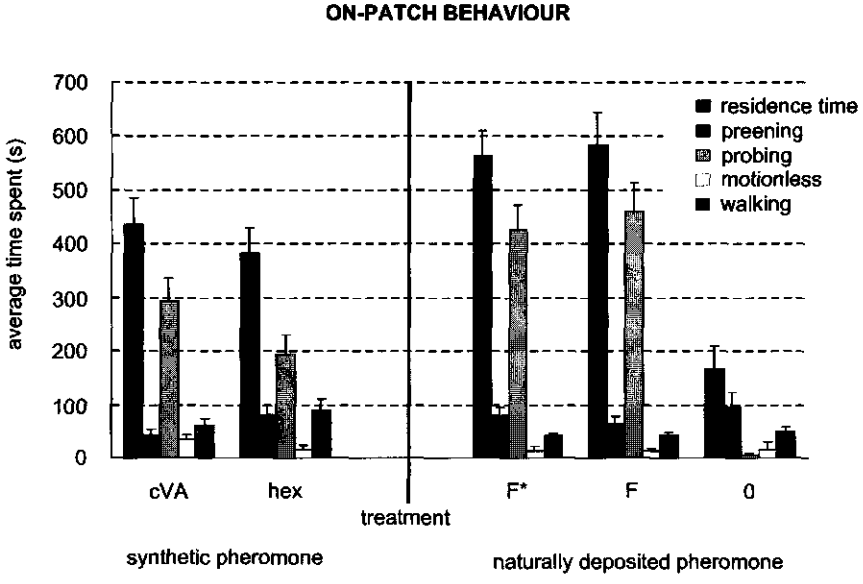

Figure 2: The average time allocation (+ standard error) to different behaviours by female *L. heterotoma* on substrate patches, treated with pheromone (cVA and recently mated flies (F*)), and on control test substrates (hexane, virgin flies (F), no flies (0)). Note that it is not possible to compare the observations of synthetic pheromone with the naturally deposited pheromones, since the patches consisted of different substrate material and the experiments were not conducted simultaneously.

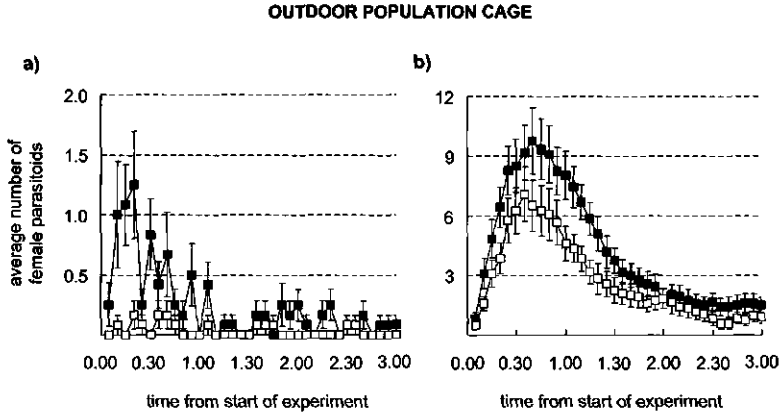

Figure 3: The average numbers (± standard errors) of *L. heterotoma* females on control substrates (□) and on substrates with aggregation pheromones (■) in population cage experiments. **a)** Synthetic pheromone: cVA versus hexane. **b)** Naturally deposited pheromone: Mated flies (FM) versus virgin female flies (F); eight larvae were added to the substrates.

rearing was high (45 %). The numbers of faecal droppings and (removed) eggs did not add significantly to the explanatory value of the model.

Field experiments

In almost all the field experiments, the cumulative numbers of*L. heterotoma* were significantly larger on substrates with pheromone than on control substrates (table 3, fig. 4). The only exception was the experiment with naturally deposited pheromone by 24 flies (experiment 3, fig. 4c), but with higher densities of fruit flies (50-200) during incubation we found again similarly increased cumulative numbers on pheromone-treated patches compared to control patches (experiment 3, fig. 4d, GLM, statistical trend, $p=0.065$). The response of the parasitoids was positively dose dependent (experiment 5, fig. 4f, GLM, $p<0.001$). The raised cumulative numbers of parasitoids on substrates with hosts (fig. 4a *vs* 4b) was probably caused by the arrestment of the parasitoids. In experiment (2) and (5), the fraction of parasitized larvae was significantly higher in the pheromone-treated substrates than in the control substrates (experiment 2 and 5), even showing positive dose-dependence; in experiment (3) and (4), we found no significant differences in these fractions. For the different densities of*D. melanogaster* larvae (experiment 6), no significant differences were found in the fractions of parasitized larvae (GLM: $p>0.05$).

The block factor 'day' was significant in many analyses, but day * treatment interactions were not, indicating differences in daily numbers of parasitoids, but not in qualitative patterns. The co-variable 'numbers of fruit flies that visited the patches' was significant in most analyses and often confounding with the pheromone treatment factor. That can be partly attributed to the similarity in responses of parasitoids and fruit flies in most experiments (fig. 5, see below) and reflects the presence of pheromone through the (correlated) response of fruit flies, rather than a genuine effect of presence of fruit flies. In some cases,

Table 3: The effect of *D. melanogaster* aggregation pheromone on the behaviour and parasitism of larval parasitoids, *Leptopilina* spp, in population cage and field experiments. With Generalized Linear Models, we compared the cumulative numbers of female parasitoids and the fractions of parasitized larvae for pheromone-treated and control substrates. The 'Factors of Difference' and 'Odds Ratio' describe the ratio in numbers and odds, attributable to the presence of pheromone; 95 % Confidence intervals (95% CI) are given for both measures. The average percentage of larvae parasitised (and 95% CI) are calculated on angular transformed data and back-transformed. The pheromone dose in field experiment (5) was log-transformed and tested as a continuous linear (L) and quadratic (Q) predictor. Significance of Factors of Difference and Odds Ratios is denoted by: *** ($p<0.001$), ** ($p<0.01$), * ($p<0.05$), " ($p\leq0.065$) or ns ($p>0.065$). For further explanation, see materials and methods.

experiment	number of parasitoids	percentage of larvae parasitized		
	Factor of Difference (95% CI)	average % (+ 95%CI) pheromone	control	Odds Ratio (95% CI)
Population cage experiments				
1) synthetic: cVA vs hexane	7.47 (2.63-21.17) ***	-	-	-
2) naturally deposited: FM vs F	1.54 (1.06-2.25) *	43 (25-62)	46(24-69)	ns
Field experiments				
1) synthetic: cVA vs hexane, discs	2.76 (1.12-6.82) *	-	-	-
2) synthetic: cVA vs hexane	2.62 (1.27-5.37) **	61 (46-76)	33(15-54)	2.77 (1.14-6.74) *
3) naturally deposited: FM vs F	ns	58 (38-78)	65(40-85)	ns
naturally deposited: FM vs F, high densities	3.11 (0.85-11.48) "	-	-	-
4) fly traces + synthetic: F, cVA vs hexane	2.40 (1.46-3.95) ***	72 (52-89)	51(32-70)	ns
5) doses of cVA, vs hexane	L: 2.18(1.44-3.29)***			L: 1.22(1.01-1.46)*
0.45 ng cVA	Q: 0.86(0.78-0.94)**	40 (27-53)	31(13-52)	Q: ns
4.5 ng cVA		39 (26-51)		
45 ng cVA		56 (34-76)		
6) different larval densities,	-			ns
cVA 4		31 (5-66)	-	
8		16 (2-38)		
16		36 (11-66)		
32		54 (55-62)		
7) control experiment: re-newing	3.52 (1.93-6.40) ***	-		-

however, the co-variable added considerably to the explanatory value of the model, and the fruit flies that visited the patch during the experiment contributed to the attractiveness of these patches. Likewise, we found a positive relationship between the numbers of parasitoids and fruit flies on the petri dishes with hexane (GLM, $p<0.01$).

The control experiment (7), in which the substrate patches were refreshed and re-located to diminish the effects of fruit fly presence, showed similar results as the other experiments (fig. 4). The co-variable 'numbers of fruit flies' was a confounding variable in this experiment, and did not further contribute to explanatory value. The responses of parasitoids and fruit flies were strikingly similar in most experiments (fig. 5).

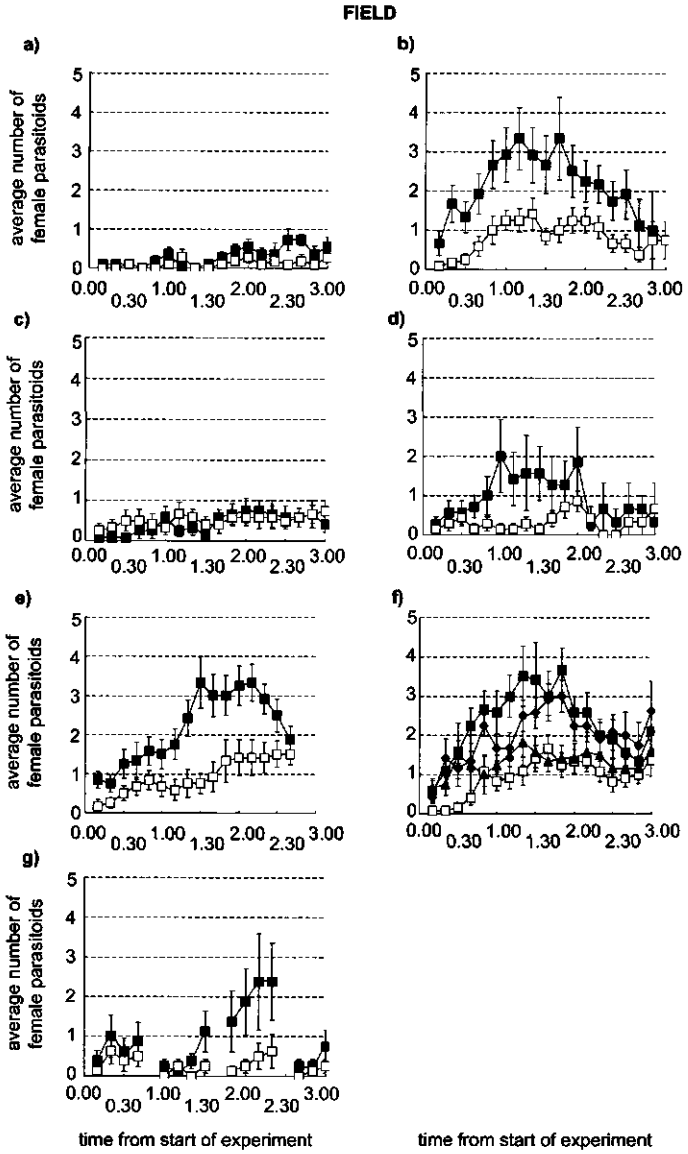

Figure 4: The average numbers (± standard errors) of female *Leptopilina* spp. on control substrates (□) and on substrates with aggregation pheromones (■) in field experiments. **a)** experiment 1: synthetic pheromone cVA versus hexane (discs, no hosts). **b)** experiment 2: Synthetic pheromone: cVA versus hexane. **c)** experiment 3: Naturally deposited pheromone by 24 mated flies (FM) versus 24 virgin females (F). **d)** experiment 3: Naturally deposited pheromone by 50–200 mated flies (FM) versus 50–200 virgin females (F). **e)** experiment 4: All substrates incubated with virgin females (F), synthetic cVA versus hexane. **f)** experiment 5: Different doses of synthetic pheromone: 45 ng (◆), 4.5 ng (■), 0.45 ng (▲), hexane (□). **g)** Control experiment (7) with synthetic pheromone cVA versus hexane, substrate patches renewed every 40 minutes. See also table 1 and materials and methods for further information on the experiments.

The occurrence of super- and multiparasitism (experiment 8) was found in approximately 10 % of the parasitized larvae, with 2 parasitoid eggs in a drosophilid larva on 5 occasions, and 3 parasitoid eggs on 1 occasion. All fruit fly larvae with multiple parasitoid eggs came from pheromone-treated substrates. The percentage parasitism when test substrates had been exposed for 3 hours (experiment 9) was only slightly lower than after 15 hours of exposure, indicating that the majority of parasitizations occurred during the experiments, and patterns of parasitism were therefore closely related to the observed behavioural responses.

Discussion

In this study, we investigated the effects of espionage in the complex of parasitoid behaviours when searching for host, taking the dynamic use of information in consideration. During host searching by *L. heterotoma*, substrate selection is strongly affected by the presence of *D. melanogaster* aggregation pheromone, but after arrival on a patch, aggregation pheromone is of minor importance. In the laboratory we found strong chemotactic responses of *L. heterotoma* to substrates with aggregation pheromone of adult fruit flies, as was previously shown (Wiskerke et al. 1993a, Hedlund et al. 1996a). Substrate quality further determined the strength of the chemotactic response towards the aggregation pheromone. The residence and probing time on substrates with adult fly traces was strongly prolonged, compared to substrates

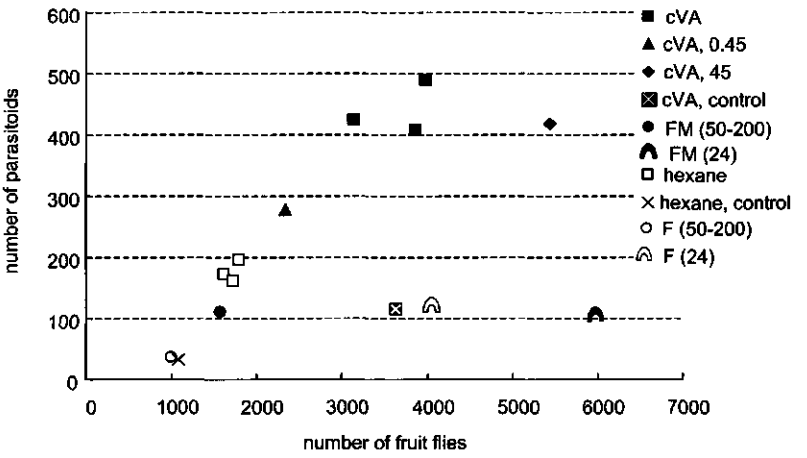

Figure 5: Similarities in responses of adult fruit flies and parasitoids to substrates in the field experiments. Each symbol corresponds to the total numbers of fruit flies and parasitoids, summed over all replicates per experiment. The totals for the cVA and hexane test substrates were derived from experiment (2, 4 and 5), the different cVA doses from experiment (5), FM and F from experiment (3) and the 'cVA, control' and 'hexane, control' from the control experiment (7) (see table 1 and methods for further explanation).

without fly traces, but the aggregation pheromone played no significant role within the complete blend of adult fly traces. Also the numbers of host in a patch prolonged searching and residence time, but again, the presence of aggregation pheromone had no effect. Evidently, the parasitoids switched to other cues, presumably more reliable ones, after arrival on a substrate. In the population cages and in the field, we found again clear chemotactic responses in substrate selection by *Leptopilina* spp. when these contained *D. melanogaster* aggregation pheromone. Furthermore, the numerical response of the parasitoids was positively dose-dependent. When no larvae or adult fly traces were present on the pheromone-treated substrates, the parasitoids left quickly (fig. 3a and 4a). For most experiments, the responses of parasitoids largely matched those of the fruit flies. In several field experiments, the fraction of fruit fly larvae that was parasitized was higher in substrates with aggregation pheromone than in control substrates. This reveals an ecological cost to the use of aggregation pheromones in adult *D. melanogaster*, in terms of an increased risk of parasitism for their larvae.

From the parasitoids' perspective, the aggregation pheromone of *Drosophila* is a reliable cue for host habitat location, because the pheromone is linked to oviposition behaviour of the fruit flies (chapter 3). The parasitoids refine their responses by a number of mechanisms: 1) They only react to the pheromone when substrate quality is sufficiently high, 2) they increase their response to higher doses of pheromone and 3) they respond to the microdistribution of the pheromone. It is noteworthy that the responses by parasitoids to aggregation pheromone corresponded largely to the responses by fruit flies: Fruit flies also respond in higher numbers on high quality substrates (chapter 4), they have positively dose-dependent numerical responses, the microdistribution of egg deposition within substrates is correlated to the microdistribution of pheromone, and increasing adult fruit fly densities yield increasing numbers of fruit fly eggs (chapter 3). Therefore, the chemotactic response of the parasitoids are in full accordance with the accuracy criterions. With their behavioural responses, parasitoids quantitatively differentiate between patch profitabilities at long range, thereby reducing waste of time in non-profitable habitats. A similar capability of long range, quantitative assessment of patch profitability was found for a parasitoid that uses volatiles from a herbivore-attacked plant as a third-party information source (Geervliet et al. 1998). These responses of parasitoids to variation in host density may have profound influences on population dynamics of hosts and parasitoids (e.g. Hassell 1982, Godfray and Pacala 1992, Ives 1992, Hassell 2000). Moreover, for the individual fitness of parasitoids, quantitative assessment of the environment is of immense value for obtaining a maximal efficiency, and accuracy should generally be considered as a fundamental aspect of reliability.

The quality of the substrate, here manipulated to obtain different yeast concentrations, was a decisive factor in the chemotactic behaviour of the parasitoids: *L. heterotoma* was only attracted to the aggregation pheromone in substrates with relatively high concentration of yeast. It seems likely that this conditional response to the pheromone is adaptive, since the survival of the parasitoid is largely dependent on the survival of the host, and increased concentrations of yeast enhance the survival probabilities of hosts, especially at high larval densities (Bakker 1961, B. Wertheim, unpublished results).

In the field experiments with naturally deposited pheromone, deposited by low densities of fruit flies, we found no significant responses of the parasitoids. Increasing the numbers of fruit flies during

incubation resulted in similar differential response patterns of the parasitoid as for the synthetic pheromone. Possibly, the amount of deposited pheromone by fruit flies was low, compared to the pheromone concentrations on the surrounding (naturally occurring) substrates. Fruit fly densities on resources in France were much higher than the 24 flies that we initially used during incubation (see also chapter 3).

The on-substrate host location behaviours were based on other cues than the presence of aggregation pheromone. Since female fruit flies can only obtain the pheromone through mating (Butterworth 1969, Bartelt et al. 1985b), which is costly to females in terms of survival (Chapman et al. 1995), and the females are subsequently likely to exhaust the pheromone before they deplete the sperm, it seems appropriate for a parasitoid to rely more on adult traces than on the presence of pheromone. Additionally, the presence and density of larvae appeared important factors in the parasitoids patch time allocation (see also Vet 1985, Haccou et al. 1991, Vet et al. 1993). We found no evidence for any further effects of aggregation pheromone on patch time allocation. *Leptopilina heterotoma* can detect the presence and density of larval hosts by host-derived kairomones (Dicke et al. 1985), and furthermore uses a patch leaving decision mechanism that is based on encounters with hosts and ovipositions (Haccou et al. 1991, chapter 7). Since such direct information has the highest reliability and accuracy, the lack of response to aggregation pheromone in this situation is also in accordance with the accuracy criterion.

Our findings all support the notion that information use by parasitoids is highly dynamic and the full array of stimuli present in natural situations should be considered. By switching from cue to cue during different phases in the foraging process, *L. heterotoma* can use the most accurate information that is available to her. Flexibility is even further employed by her ability to learn (Vet and Schoonman 1988, Vet and Groenewold 1990, Vet et al. 1990). After an oviposition experience on a certain substrate type, *L. heterotoma* exhibits increased attraction to that substrate type, and shorter travel times by more direct travel paths towards it (Papaj and Vet 1990, Vet and Papaj 1992). It is yet unknown what role aggregation pheromones play in substrate selection, after a successful experience on a certain substrate type. On the basis of the reliability criterion, we expect that the 'ranked' stimulus (*sensu* Vet et al. 1990) of the pheromone after learning depends on the encounter rate with substrates that contain eggs but no pheromone, and additionally on the frequency of competition among parasitoids. In France we found evidence for competition among parasitoids from the relatively high rate of super- and multiparasitism (10 %), especially in pheromone-treated substrates. Yet, such population characteristics are likely to vary geographically, and therefore we expect regional differences in employment of a strategy that combines learned responses and responses to host aggregation pheromone.

From the fruit fly perspective, the espionage by the parasitoid constitutes a significant ecological cost to the use of aggregation pheromones, because the *D. melanogaster* larvae in substrates with aggregation pheromone were at a higher risk of parasitism from *Leptopilina* spp. This increased risk was directly related to the pheromone dose. In our studies, we only worked with low larval densities. A next step would be to combine our results on numerical responses with data on functional responses of the parasitoid. In a forthcoming paper (chapter 7), we will present a theoretical study on the combined density-dependent processes in the interaction between parasitoids and hosts: Numerical responses, mediated by the

pheromones, and functional responses, leading to a 'selfish herd' effect (*sensu* Hamilton 1971) can become opposing selective forces.

In this paper, we have highlighted how the use of aggregation pheromone affects a parasitoid-host interaction and can incur ecological costs to the host. To fully understand the impact of communication by aggregation pheromones on the ecology of *D. melanogaster*, the whole complex of costs, benefits and constraints should be taken into account (Dicke and Sabelis 1992). Such an ecological cost-benefit analysis requires the inclusion of the full web of intra- and interspecific interactions that are influenced by the use of aggregation pheromone.

Acknowledgements

Mr. Marboutie, Freddy Combe and Vincent Mercier from the INRA field station in Gotheron are gratefully acknowledged for allowing the field experiments on their premises and providing accommodation and research facilities. Michel Boulétreau, Roland Allemand and their research group are thanked for valuable discussions and kind hospitality. René Boom, Kees Sol, Rob Nout, Willy Herder and Anja van Aggelen helped in designing and constructing the experimental set-ups. Gerrit Gort guided the statistical analyses. Henk Snellen en Leo Koopman helped in rearing the insects. The PhD discussion group gave valuable comments on the manuscript. Funds from SIR-NWO, PICS and the Uyttenboogaart-Eliasen Foundation enabled the field study in France.

CHAPTER 7

Individual risk of parasitism in host aggregations: A behaviour-based model on functional and numerical responses

Abstract

Aggregative behaviour in animals has frequently been explained in terms of a *per capita* diluted risk of attack from natural enemies. Reduced risk, however, could be opposed by a variety of behavioural responses of natural enemies to the density of their victims. In this paper, we examined the combined effect of numerical (or aggregative) and functional responses of insect parasitoids on the risk of parasitism in host aggregations. To determine whether and when aggregation is beneficial for a host with respect to risk of parasitism, we developed a partly mechanistic, partly descriptive model, using data from behavioural experiments in the laboratory and field on *Leptopilina* - *Drosophila* interactions. We modified the parasitoid function in the classic Nicholson Bailey model, to include the behavioural responses of parasitoids to host-originating cues (aggregation pheromones) and a flexible patch leaving decision rule, described by an incremental effect of ovipositions on searching time. The model predicts no dilution of risk at high host densities, but instead an ever increasing risk of parasitism. This is not caused by the numerical response, but by the flexible time allocation of the parasitoids. The numerical response towards the aggregation pheromone augmented the risk across *all* host densities. Therefore, the use of aggregation pheromones constitutes an ecological cost with respect to risk of parasitism, while the benefits of aggregative behaviour itself have to be sought in other directions than a diluted risk of parasitism. The qualitative prediction of the model, i.e., increased risk of parasitism with increased host densities, was highly robust, but quantitative predictions on the number of hosts attacked were sensitive to a variety of model parameters. A field experiment on risk of parasitism yielded the predicted qualitative pattern of parasitism, but differed considerably from the quantitative predictions. Several potentially responsible processes are discussed from an ecological and evolutionary perspective.

Introduction

Since Hamilton (1971) introduced the concept of a selfish herd, by illustrating that individuals in an aggregation experienced a diluted risk of attack from natural enemies, aggregative behaviour has frequently been presumed to serve the avoidance of predation (Bertram 1978, Simser and Coppel 1980, Elnaiem and Ward 1991, Parrish and Edelstein-Keshet 1999, Hunter 2000). Hamilton implicitly assumed a quick satiation in the predator and no changes in predator density in response to prey densities. In reality, however, many natural enemies can flexibly adjust their foraging behaviour to exploit increased densities of their victims (Turchin and Kareiva 1989, Wrona and Dixon 1991). To further our understanding on costs and benefits for individuals within aggregations, with respect to risk of attack from natural enemies, it is essential to include the suite of behaviours of natural enemies that, together with prey behaviour, determine such risk of attack (Vet 1996, Bernstein 2000, Casas 2000).

The two density dependent behavioural responses that are central to parasitoid-host interactions are the numerical (also known as aggregative) response and the functional response. The numerical response of parasitoids describes the numbers of individual parasitoids that move into the habitat (Solomon 1949, Holling 1959). Parasitoids can use an array of cues to estimate habitat profitability (van Alphen and Vet 1986, Godfray 1994), which often results in an increased parasitoid load in habitats with high host densities. The functional response of the parasitoids describes the numbers of hosts that are attacked at different host densities per unit of time (Solomon 1949, Holling 1959). Many parasitoids experience a decreased efficiency at high host densities, due to (re)encounters with parasitized hosts and handling time effects (Nicholson and Bailey 1935, Holling 1959). This could potentially lead to a *per capita* diluted risk of parasitism in host aggregations. Yet, also during this phase of host searching, the parasitoid uses cues and experiences to adjust her behaviour to local host densities. To predict the individual risk of parasitism for hosts, one has to combine the effects of higher parasitoid loads on aggregations, the reduced efficiency in such aggregations, and behavioural flexibility in response to host cues. Although the classic models on functional responses include a parameter for numerical responses (usually denoted as 'P'), these models are not very flexible (e.g., fixed residence times or alternatively, fixed numbers of parasitoids per substrate patch (Comins and Hassell 1979, Hassell 1982, Wellings 1991, but see also Hassell and May 1974) and do not capture the complex of behavioural responses of parasitoids (Vet 1996).

The cues that parasitoids use during all phases of host searching, can originate directly from the hosts, or from 'third parties', e.g., plants or other host stages than the one under attack (Vet and Dicke 1992, Stowe et al. 1995, Haynes and Yeargan 1999). The extent to which the different information sources are used by parasitoids is determined by their detectability, their reliability (i.e., the connection between a cue and the presence of the vulnerable host stages) (Vet et al. 1991, Vet and Dicke 1992) and their accuracy (e.g., the link between cue dose and host density) (chapter 6). The different information sources are used by parasitoids for both habitat selection and within-habitat decision rules (see chapter 6 for a more thorough discussion).

One especially intriguing source of information that parasitoids may use during host searching, is the host's aggregation pheromone. Aggregation pheromone is emitted by an individual and induces aggregative behaviour in conspecifics at the locality of release. These pheromones comprise a magnificent opportunity for parasitoids to exploit, because they 'announce' host aggregations and they can realise the detectability, reliability and accuracy that is desired by the parasitoid. At the same time, however, they do induce the aggregative behaviour that might shield hosts from parasitism by a 'selfish herd effect' (Hamilton 1971). Thus, the pheromone can induce a numerical response of the parasitoids but at the same time the high host densities can dilute the *per capita* risk of parasitism, i.e., numerical and functional responses become opposing forces. Whether, and at what densities, aggregation yields a net benefit to the individuals with respect to attack from parasitoids, depends on the shape of the functional and numerical response of the parasitoids. These shapes in their turn, depend on the behaviour of the parasitoids.

To investigate this complex issue, we study the *Drosophila* – *Leptopilina* interaction. In the fruit fly *Drosophila melanogaster* Meigen, adults emit volatile aggregation pheromones (Bartelt et al. 1985b), that induce aggregated ovipositing in female fruit flies (chapter 3 and 4). The larval parasitoids *Leptopilina heterotoma* (Thomson) and *L. boulardi* (Barbotin et al) eavesdrop on the adult fruit flies' pheromone to localize their larval hosts (Wiskerke et al. 1993a, Hedlund et al. 1996a, chapter 6). Substrates with aggregation pheromone received larger numbers of parasitoids in field experiments and this numerical response was positively related to the dose of the pheromone and to the numbers of fruit flies that left traces at the substrates (chapter 6). Direct mutual interference (i.e., interactions between parasitoids that reduce searching efficiency) is thought to be insignificant for this parasitoid species (Visser and Driessen 1991). The functional response of *L. heterotoma* shows a slightly decelerating curve in the laboratory (this paper).

To assess whether and when aggregation is beneficial for individual hosts with respect to risk of parasitism, we developed a simple model on parasitoid behaviour, that is partly mechanistic and partly descriptive. Its purpose is to predict the risk of parasitism for individual hosts across a realistic range of host-densities. The model is based on the classic Nicholson Bailey model, and we included the behavioural responses of parasitoids to densities of hosts through the effects on habitat selection (i.e., the response to the host's aggregation pheromone) and on searching times within habitats. Searching time was not fixed, but was defined by a patch leaving decision rule that described the incremental mechanism: the motivation to keep searching on a patch is increased in response to ovipositions (Waage 1979). The incremental mechanism ascertains that we do not have to pre-define a fixed time window or giving-up-time, and assumes that patch leaving is based on experience, as seems just for *L. heterotoma* (Haccou et al. 1991). For parameterization of the model, we used combined laboratory and field data on the behaviour of *D. melanogaster* and its larval parasitoids *L. heterotoma* and *L. boulardi*. After evaluating the basic model, two other density dependent processes were included in the model to assess the sensitivity of the model to these processes. Firstly, after arrival on a substrate, *L. heterotoma* can estimate substrate profitability through larval traces or kairomones (Dicke et al. 1985). This also affects searching time (Vet 1985). Secondly, Visser (1990) showed that *L. heterotoma* increased residence time at higher parasitoid densities because of superparasitism. Superparasitism is the acceptance of already parasitized hosts.

Females more readily accepted parasitized hosts when searching in groups, and therefore they stayed longer on substrate patches. The sensitivity of the basic model to the model parameters was assessed with Monte Carlo simulations. The predictions of the model were validated with the results of a field experiment (described in chapter 3).

Methods

FUNCTIONAL RESPONSE

Experimental procedure

To quantify the functional response of the parasitoids, the behaviour of individual *L. heterotoma* females was observed on artificial substrate patches with different numbers of second instar *D. melanogaster* larvae (0, 1, 2, 4, 8, 16 or 32 hosts). The larvae were introduced 1-3 hours before the experiments started. *Leptopilina heterotoma* searches for hosts by walking randomly across a substrate during which she continuously and rhythmically probes the substrate with her ovipositor (Vet and Bakker 1985). She finds a host by hitting it with her ovipositor (Vet and van Alphen 1985). If she accepts the host (insertion of ovipositor for > 20 s, clearly visible during observation), oviposition occurs and afterwards she almost immediately continues searching for other hosts.

One day before the experiments, groups of 10 female wasps were incubated for half an hour on a thin layer of yeast with second instar *D. melanogaster* larvae to gain oviposition experience. Subsequently, the wasps were kept in groups of 50-100 in vials (4.5 cm ⌀, 8.5 cm height) with water agar (20 g / l) and a drop of honey on the plug. Experiments started by gently introducing a single female wasp onto an artificial yeast patch, in the centre of a petri dish (5.4 cm ⌀). The patch consisted of 1 ml yeast suspension (Fermipan, Gist Brocades, 250 mg / ml water; 21 mm ⌀) on top of a layer of water agar. For each experiment, a new wasp and a new patch were used. The experiments were performed in open petri dishes. The wasp was observed for as long as she chose to remain on the patch; leaving was defined as abandoning the yeast patch for more than one minute or flying off. The numbers of ovipositions (n_a), handling time for each host (t_h = time from insertion of the ovipositor until resuming host searching behaviour), total residence time on the patch (T_t), individual searching time (T_s = time spent probing and walking on the substrate) and probing frequency (pf) were recorded for each female wasp. Encounter rates ($a' = pf \cdot A_l / A_p$, where A_l is the surface area of one larva and A_p is the surface area of the patch) were calculated. The experiments were performed by students during practicals in 5 successive years.

MODEL

Model definition

Our model is based on the classic Nicholson-Bailey model, for which parasitoid searching behaviour is described in 'Nicholson's competition curve',

$$N_a = N_t \cdot (1 - \exp(-N_e / N_t)) \quad (1a)$$

$$N_e = a' \cdot T_t \cdot N_t \cdot P \quad (1b)$$

where N_a *is* 'number of hosts attacked', N_e is 'number of hosts encountered', N_t *is* 'total number of hosts present', a' is a constant and species-specific 'encounter rate' (also known as 'area of discovery', 'searching efficiency' or 'attack rate'), T_t is 'total residence time', and P is 'number of parasitoids'. The probability for hosts to escape parasitism is given by the zero-term of the Poisson distribution, $p_{pois}(0) = \exp(-N_e / N_t)$, assuming random search by the parasitoid. Consequently, the probability for a host of being encountered at least once is given by $1 - p(0)$.

In Nicholson's competition curve, it is unrealistically assumed that parasitoids spend all their time on host-searching. Just as several other models that assume a more flexible time-spending behaviour in parasitoids (Rogers 1972, Arditi 1983), we substitute the total residence time by 'individual searching time', T_s. In contrast to common practice, however, we do not approximate searching time by subtracting total handling time (T_h) from total residence time (T_t) (see for example Rogers 1972, Hassell 1982). Our motive is that individual searching time in natural situations is mostly not determined by a fixed time window, but based on plastic behaviour resulting from patch leaving decision rules. We consider individual searching time as a function of experience, $T_s(x)$, describing a patch leaving decision rule, which we will define below. The number of host encountered in our modified model becomes

$$N_e = a' \cdot T_s(x) \cdot N_t \cdot P \quad (1c)$$

We define the model at the spatial scale of a single substrate, containing a specified number of hosts (N_t). The numbers of parasitoids and the numbers of attacked hosts are considered continuous variables. Furthermore, we define the 'numbers of host attacked per individual parasitoid', n_a, as follows

$$n_a = N_a / P \quad (1d)$$

thus assuming simultaneous depletion of a substrate by all P parasitoids.

To compare the predictions for randomly searching parasitoids with predictions for slightly clustered searching patterns of the parasitoid within substrates (i.e., area restricted search, independent of the host distribution within the patch, see 'results' for motivation), we replace the zero-term of the Poisson distribution for the zero-term of the Negative Binomial distribution: $p_{neg.binom.}(0) = [1 + (N_e / (N_t \cdot k))]^{-k}$, where k is the clumping index of the Negative Binomial distribution. Values of $k < 1$ describe a clustered distribution, while in the limit of $k \to \infty$, it approaches a random (Poisson) distribution.

Not only the individual searching time, T_s, but also the number of parasitoids, P, is in fact a function. Individual searching time is a function of the number of parasitizations, $T_s(n_a)$, through an incremental mechanism, where each oviposition raises the parasitoid's motivation so as to search longer on a patch. The number of parasitoids, P (i.e., the numerical response), is quantitatively dependent on the number

3e) and the numerical response (eqn. 4) of the parasitoids. From these functions we can numerically solve for n_a (Matlab, v. 5.2.0, fzero command) and calculate N_a and $P(N_t)$.

As it stands, the model is only an approximation based on two assumptions (see appendix): 1) The expectation of a function of n_a is the function of the expectation of n_a, which is not true in general for non-linear functions, but for this particular situation proves a very good approximation; 2) The ratio between the surface area of a substrate and the surface area of all hosts is large (»1), which applies for our parameter settings.

The individual risk for hosts in an aggregation is calculated as the percentage of hosts that is attacked (N_a / N_t). These percentages are plotted against host density (N_t), and the slope and plateau of this risk curve are described by the first ($N_t = 1$) and last value ($N_t = \max N_t$) respectively.

Sensitivity to additional density dependent processes

Two additional density dependent processes are incorporated in our basic model, to evaluate the sensitivity of the model for these processes. Firstly, the effects of larval kairomones on initial motivation are investigated. The initial motivation, M_0, in eqn. (3e) is substituted by

$$M_0(N_t) = M_0(0) + c \cdot N_t / (1 + N_t) \tag{5a}$$

where c is fitted from experiments, where *L. heterotoma* searches on patches without hosts, but with increasing concentrations of larval kairomones (Dicke et al. 1985).

Secondly, we extend the model to include superparasitism. Behavioural experiments showed increased patch residence times for each female when searching in groups, even when the patch visits were sequential (Visser et al. 1990, van Alphen et al. 1992). The mechanism behind the increased residence times is thought to be simply the increased numbers of ovipositions by each female (Visser et al. 1992). We therefore choose to treat superparasitism as additional ovipositions in the formula for the searching time with incremental mechanism. We use a pragmatical approach by changing the upper limit of the sum in the function for searching time (eqn. 3e):

$$T_s(n_a) = M_0 + \sum_{i=1}^{n_a/s} \frac{b}{i} \tag{5b}$$

where $0 « s \leq 1$, and $(1 - s) \cdot 100$ % is the expected fixed percentage superparasitism per female. This function assumes that each female oviposits a fixed small percentage of her eggs in already parasitized larvae. This might be too simplistic to attain biological realism, but we use it to evaluate the robustness of the model to this additional density dependent process.

Parameterization of the model

The models are separately parameterized for the laboratory experiments on functional responses and the validation field experiments. In the model for the laboratory experiments, only one parasitoid searches per patch ($P = 1$, $N_a = n_a$, no numerical response). A patch contains 1-32 hosts (N_t). Encounter rates ($a' = pf \cdot A_t / A_p$) and initial motivation (M_0 = GUT when n_a equals 0) were derived directly from the

functional response data, by averaging across all host densities, because no trends with host densities were observed (see results, 'functional response'). The incremental constant for the searching time was fitted from the functional response data, by calculating the weighted least squares from the predicted searching time and the average searching time across all values of n_a. The clumping index for the Negative Binomial distribution, *k*, was calibrated with a G-test for goodness of fit (Sokal and Rohlf 1995).

The numerical response in the field experiments was parameterized from the experiments that were used to assemble eqn. (4). The average densities of parasitoids (d_p) and eggs (d_e) and the matching factor in distributions (*m*) were calculated from the statistical models. A patch contained 1-250 hosts (= N_t). To parameterize the functional response in the field was more difficult, since behavioural observations inside natural substrates were impossible. We extrapolated our laboratory data to the field experiments and had to resort to crude estimations for the remainder of the parameters. The encounter rate (a') was calculated as for the laboratory experiment, adjusting the surface area of the patch to the substrate sizes in the validation experiment. Initial motivation (M_0) was crudely estimated from an experiment in a large population cage (6 x 3 x 2 m), where similar artificial substrates were offered as in the experiment for the numerical response. Parasitoids usually left these substrates without larvae within 10 minutes (chapter 6), and therefore initial motivation was set at 600 s. We used the laboratory parameters for the probing frequency (to calculate the encounter rate), the clumping index (*k*) and the incremental constant (*b*).

Sensitivity analysis of model parameters

The sensitivity of the model for the predictions of N_a, n_a, the slope and the plateau of the risk curve was determined with Monte Carlo simulations. For the predictions of N_a and n_a, a sensitivity index, S, was used to express the predictions across all host densities, relative to the predictions for the default parameter settings:

$$S(N_a) = \sqrt{\frac{1}{N_t} \cdot \sum_{i=1}^{N_t} \frac{N_a - N_{a,default}}{N_{a,default}}}$$

and likewise for $S(n_a)$. For all model parameters, a lognormal probability distribution was specified with the default value as the mean and a standard deviation of log(2) (i.e., from half until twice the default value at a linear scale). For each simulation, two independent random values were drawn from the probability distribution for each parameter, and all possible combinations were run. These simulations were iterated 1000 times. With ANOVA decomposition, the variance components for the simulated model predictions were calculated for all parameters and all parameter interactions. The sensitivity to those parameters and interactions was expressed as the contribution (in percentages) of each to the total summed variance components.

Validation experiment

The model was validated with results from a separate field experiment. A full description of the experiment is given in chapter 3. In short, holes were punctured into apples, yielding suitable oviposition sites for *Drosophila*. The apples were placed in an orchard with a large resident population of *Drosophila*

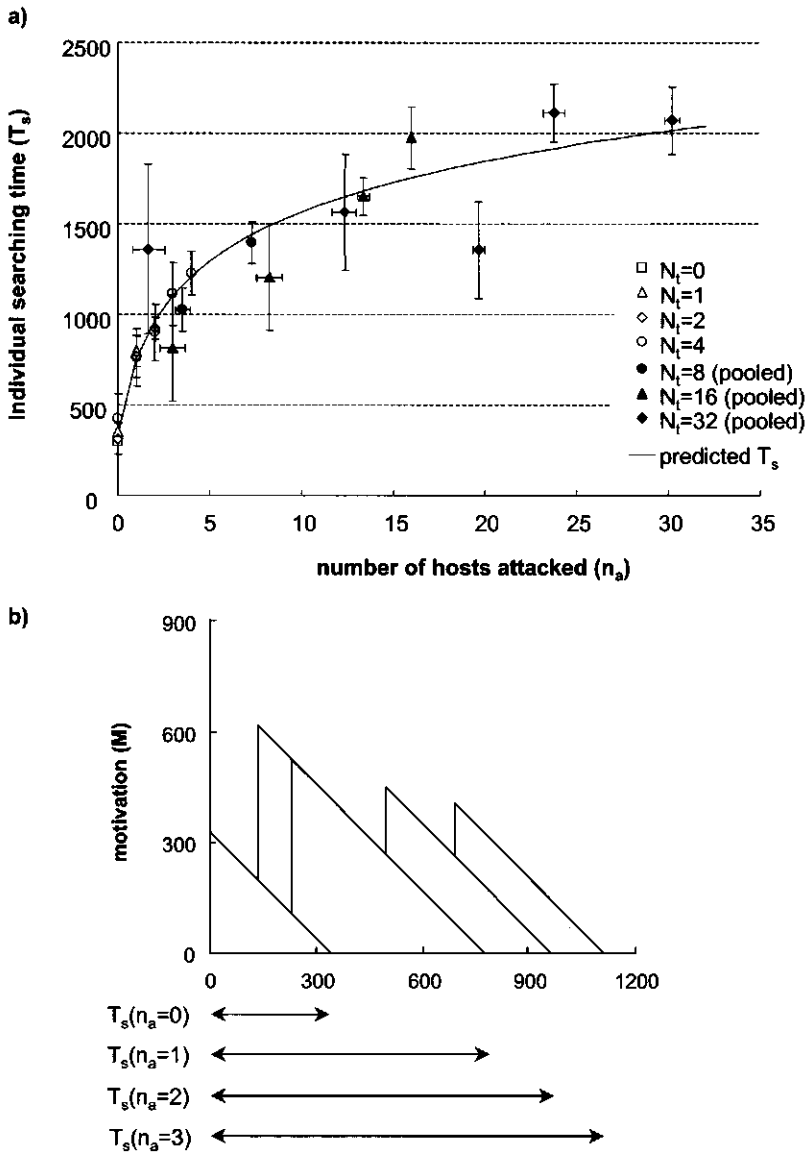

Figure 2: The average individual searching times (T_s) of *L. heterotoma* females, based on the numbers of *D. melanogaster* larvae they parasitized (n_a). **a)** Results from the functional response experiments. Different symbols indicate different numbers of host present (N_t) (see also figure 1 for explanation). Observations for host densities (N_t) of 8, 16 and 32 larvae were pooled for n_a = 0-5, 6-10, 11-15, 16-20, 21-27 and 28-32 and average searching time was plotted against the average n_a within the pool. Error bars indicate standard errors of the mean; for the pooled data also the standard errors for average n_a were calculated. The line depicts the predicted individual searching time, using the eqn. (3e), with b = 420 (see text for explanation). **b)** Graphical depiction of the incremental mechanism as described by eqn. (3e). Note that the size of the increments is not affected by the time between ovipositions (first oviposition).

MODEL

Parameterization

The parameter values for the laboratory experiments on functional responses and the validation field experiments are given in table 2. The increase in searching time with increasing numbers of ovipositions clearly followed the law of diminishing returns (fig. 2a). In our data for *L. heterotoma*, we found no evidence for a relationship between increment size on the one hand and time between ovipositions on the other hand. If such a time relationship had existed, we should have found a lower increment for the first (second, third, ...) ovipositions at high larval densities than at low larval densities, since randomly searching parasitoids at higher larval densities should on average find their hosts faster. Instead, in our data we found similar increments for the first (second, third, ...) ovipositions at each host density (fig. 2a), and only the number of previous ovipositions determined the increment sizes (fig. 2b). The incremental constant, b, was fitted from these data, and the resulting function described our behavioural data accurately (fig. 2a, drawn line).

Predictions by the model

The random parasitoid search model (eqn. 2a) was first applied to our laboratory measurements. Even though the data set was also used for parameterization of part of the model, it provided a controlled means to explore the properties of the model. Our model with random search patterns of single parasitoids (eqn. 2a; P = 1) predicted a higher risk of parasitism than we had observed (fig. 3a). Substitution of the measured individual searching time (T_s) into Nicholson's competition curve function (eqn. 1b) also overestimated the risk for hosts. The model predicted a higher number of hosts attacked at all larval densities than we had measured (fig. 3b), even approaching 100 % parasitism at the highest densities (fig. 3a). This discrepancy can be explained in two ways: 1) Searching behaviour within the patch is not fully random; 2) the encounter rate is overestimated. Both explanations are biologically realistic. Firstly, since parasitoids exhibit a marked inward turning behaviour at reaching the edge of a patch, the edge region is frequented more often and searching effort is slightly clustered instead of fully random. This applies not only for the artificial laboratory substrate patches, but also for natural substrates of finite size. Secondly, encounter rate is defined as {probing frequency} times {surface area of a larvae / surface area of the patch}. Since larvae have a tendency to burrow vertically into a substrate patch, the exposed surface area of the larvae is somewhat reduced. Both these features were incorporated in the model to evaluate their effects on the predicted risk of parasitism. A closer fit was indeed obtained by either decreasing the encounter rate by a half ($a' = 0.0009$) or by assuming a slightly clustered searching pattern for the parasitoids (eqn. 2b, $k = 0.95$, derived by calibration) (figs. 3a, b). In all cases tested, the model predicted an increase in risk of parasitism at first, levelling off at higher densities, but never a decrease at high host densities.

When the model was parameterized for the field observations (table 2, P as function of N_t (eqn. 4)), the predictions for the random searching pattern with default encounter rate again predicted almost 100 % parasitism at the highest host densities, although the steepness of the curve at low density was less

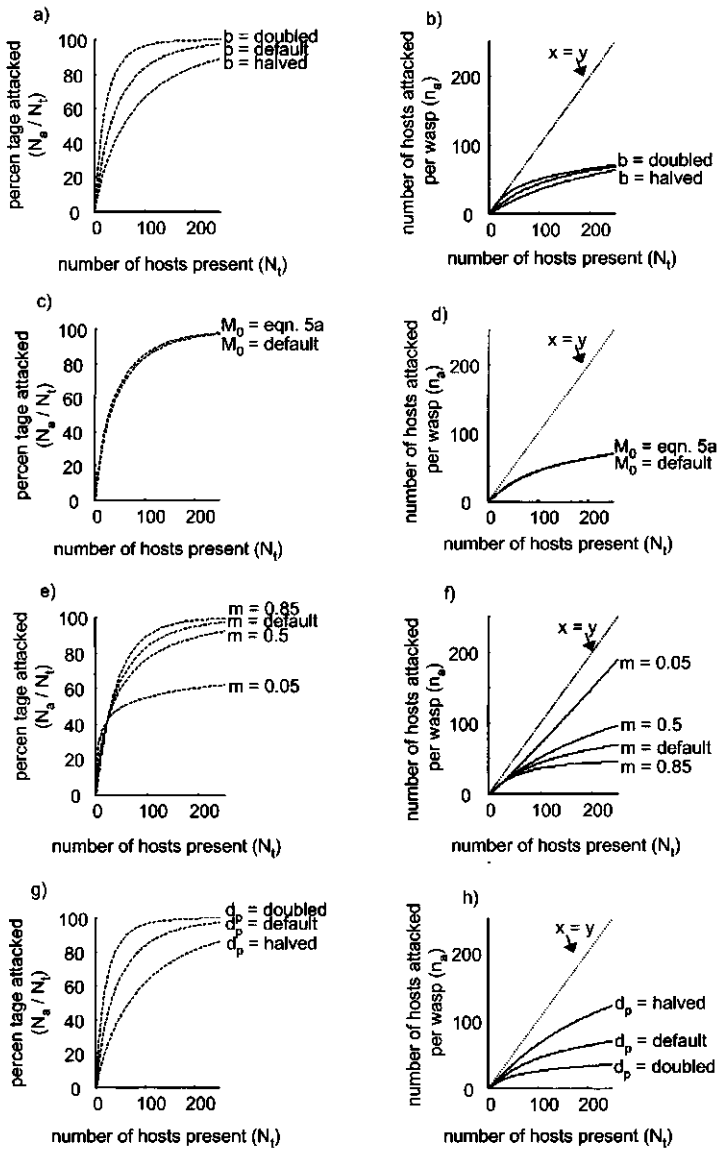

Figure 4: The sensitivity of the model predictions for the combined effects of functional and numerical responses of *Leptopilina* spp, parameterized for the field environment. The parameters that were varied were **a–b)** the incremental constant, *b*, of individual searching time (see eqn. 3e); **c–d)** initial motivation, M_0, in response to host density (see eqn. 5a); **e–f)** match, *m*, in the regression coefficients of parasitoids *vs* flies and ovipositions *vs* fly (eqn. 4); **g–h)** density of parasitoids, d_p (eqn. 4). The first column denotes the percentages (N_a / N_t) of hosts attacked by all $P(N_t)$ parasitoids and the second column the numbers of hosts attacked per parasitoid (n_a) at different host densities (N_t). The predictions were assessed for parasitoids with a random searching pattern (eqn. 2a) within substrate patches.

The initial motivation (M_0) played only a minor role (table 3). When initial motivation was varied with density of hosts according to eqn. (5a), the difference in numbers and percentage of hosts attacked was only marginal (fig. 4 c-d; $S(N_a) = 0.07$). Superparasitism (eqn. 5b) had essentially no effect on the individual risk of parasitism (for $s = 0.95$: $S(N_a) = 0.01$; for $s = 0.85$: $S(N_a) = 0.04$). For the field situation, the degree of clumping (k) in the clustered searching pattern of the parasitoid had little effect on numbers of hosts attacked across all host densities, except the highest (table 3).

Validation of the model

The data of the field experiment with punctured apples showed a large variation in the numbers and percentages of *Drosophila* spp. larvae that were parasitized by *Leptopilina* spp. By calculating means and standard deviations of pooled groups of values, and superimposing those on the raw data, a pattern emerged that was qualitatively in agreement with our model predictions (fig. 5). The numbers and percentages of larvae that were parasitized increased monotonically, and also the level-off in risk of parasitism to a plateau seemed to occur (fig. 5a). Both the numbers and percentages of attacked hosts increased significantly with larval density (GLM, numbers: Poisson distribution, Log link, $p < 0.001$; percentages: Binomial distribution, Logit link, $p < 0.05$). The size of the substrates did not significantly contribute to the explanatory value of the statistical model. Quantitatively, the rates of parasitism in our field experiments were much lower than predicted by the model.

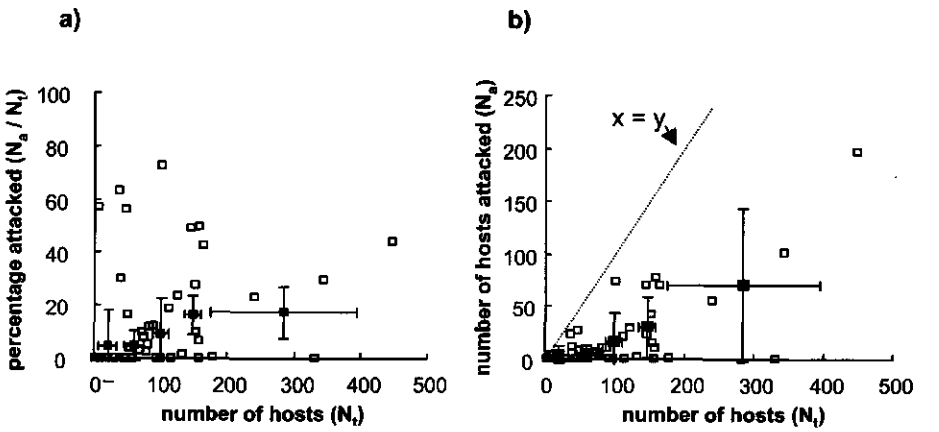

Figure 5: The number and percentage of host attacked in the validation experiment. Apples were placed for one week in an orchard with resident population of *Drosophila* spp and *Leptopilina* spp, and then insects were reared from each fruit individually. The emerged flies and parasitoids were summed to estimate the total number of hosts present (N_t), and the emerged parasitoids represent the number of host attacked (N_a). Open symbols represent the data of one apple. The solid symbols represent averaged values for pooled data of apples with larval numbers (N_t) of 0-40, 41-80, 81-120, 121-160 and >160. **a)** Average percentages ± standard deviations (back transformed values after angular transformation of raw data) and **b)** average numbers ± standard deviation were plotted against average host numbers (N_t) per pool ± standard deviations.

Discussion

Our model predicts that in the *Drosophila* - *Leptopilina* interaction, the combined effects of the functional and the numerical response result in a monotonically increasing, saturating risk of parasitism with increasing host density. Thus, the model predicts that at the scale of single substrates, aggregation is not beneficial for individual hosts with respect to risk of parasitism. The monotonic increase is caused by the flexible patch leaving behaviour of the parasitoid, that adjusts individual searching times to host densities, according to an incremental mechanism. This patch leaving mechanism on its own is a sufficient condition for the increased risk at increasing host densities, as was also found for another drosophilid larval parasitoid, *Asobara tabida* (van Alphen and Galis 1983). Several other studies on functional responses at a behavioural time scale also find that especially searching time is important in determining the risk of parasitism (Hertlein and Thorarinsson 1987, Casas et al. 1993, Ives et al. 1999). The dose-dependent numerical response of the parasitoid to the fruit fly aggregation pheromone is not required for the monotonic increase in risk, but does have an augmenting effect on the numbers and percentages of hosts that are attacked across *all* host densities.

Thus, the behavioural flexibility of the parasitoid precludes a beneficial dilution effect for clustered larvae with respect to risk of parasitism. This is in contrast to the frequent assumption that clustered distributions serve to avoid predation. Furthermore, the use of aggregation pheromone by adult fruit flies constitutes a cost in terms of the survival probability of their offspring, because of the numerical response of the parasitoids. The increase in risk with increasing density is fastest at low larval densities, and levels off to a plateau at high host densities. Therefore, the cost stabilizes after reaching a certain density, which might allow for further expansion of the aggregation size. Why larvae are aggregated cannot be explained by diluted risk of parasitism, but might be related to an Allee effect in communal resource exploitation (see chapter 2, 5).

Clearly, the outcome of our model does not apply to all natural enemies, but depends very much on the characteristics and behaviour of the specific natural enemy. For example, Fels et al. (1995), Wrona and Dixon (1991) and Turchin and Kareiva (1989) found diluted risk from predators in prey aggregations, even though the numerical response of these predators also increased at high prey densities. Conversely, Hieber and Uetz (1990) found an overall increased risk of parasitism in larger colonies of spiders, but the contributions of different parasitoid species varied due to differences in their behaviours. Our study illustrates again the importance of including the variety of relevant individual behaviours in studies on the interactions between organisms and their enemies.

The results of our validation field experiment on risk of parasitism in natural substrates corresponded qualitatively with the model predictions. Although the variation in our field data was large, the predicted pattern could be discerned. We found an initial significant increase in risk with host density and this risk seemed to level off to a plateau for high host densities. The slope of the curve and the height of the plateau, however, were considerably lower than predicted by the model. Since the model is quantitatively sensitive to a variety of parameters, a variety of processes and incorrect parameterizations can create the

discrepancy (see below). Additionally, the searching pattern of the parasitoid within substrates might be slightly clustered, since the finite size of substrates restrains the movement of a parasitoid somewhat, causing deviations from fully random search. Apart from these parameter-linked processes, a number of other processes is left out of the model, but could also have operated. For example, *D. melanogaster* larvae are known to defend themselves by encapsulating parasitoid eggs (Rizki and Rizki 1984), thus leading to an under-estimation of parasitism when judged on the basis of parasitoids that emerge. Furthermore, we modelled the deposition of eggs and aggregation pheromone by fruit flies as instantaneous and discrete from the subsequent numerical response of the parasitoids, while in reality egg- and pheromone-deposition and parasitoid searching behaviour overlap in time. In addition, exploitation by parasitoids might have been partly sequential rather than simultaneous (Lessels 1985). Egg-limitation in the parasitoids (Lessels 1985, Driessen and Hemerik 1992, Ellers et al. 2000) and adverse weather conditions (Weisser et al. 1997) could have moderated parasitism and might have modified the behaviour of the parasitoid. Overall, we did not include individual variation in behaviour and physiological state for parasitoids (Vet 1996), variation between hosts (Ives et al. 1999) and differences in host species suitability (Carton et al. 1987, Janssen 1989) and in the searching behaviour of *L. heterotoma* and *L. boulardi* (Vet and Bakker 1985, Vet et al. 1993). To incorporate all these factors would necessitate a complex individual-based model. We believe, however, that the processes we have taken into account capture the essence of the problem, making our qualitative predictions robust, while other factors will only change the quantitative predictions.

Sensitivity of the model

Although the qualitative predictions of the model were very robust, and applied for all investigated parameter settings, the quantitative predictions were sensitive to a range of parameters and to the searching pattern of the parasitoid. The parameters that influenced the quantitative predictions were encounter rate (a'), the incremental constant (b), the density of parasitoids (d_p) and the degree of matching between parasitoid numbers and fly ovipositions (m). Changes in the initial motivation (M_0) and density of fruit fly eggs (d_e) had little effect. When the searching pattern was assumed to be slightly clustered within substrates, a larger percentage of hosts was predicted to escape parasitism and the degree of clumping (k) in the parasitoid's search pattern also influenced this percentage somewhat.

The large sensitivity of the model to the encounter rate of the parasitoid indicates that encounter rate is an important handle for selection to work on. The vertical burrowing of hosts reduces their exposed area towards parasitoids, and this can largely reduce risk of parasitism. For a related *Drosophila* species breeding in rowan berries, it was hypothesized that aggregation might have evolved because communal burrowing might prove more efficient in attaining a partial refuge against parasitoids (Hoffmeister and Rohlfs 2001). Although the experimental data did not support the hypothesis on positive density dependence, they did support the existence of a refuge from parasitoids in 3-dimensional environments. The risk of parasitism in rowan berries levelled of at much lower values (40 %) than for the same parasitoid in 2-dimensional artificial substrates (van Alphen and Galis 1983). The two genetic movement strategies that are known for *D. melanogaster* larvae, i.e., 'sitter' and 'rover' (roamer) were also associated with their effect on the encounter rate of natural enemies (Sokolowski and Turlings 1987). In fact, apart

from direct defence mechanisms such as encapsulation, the reduced exposure might be the only opportunity for larvae to reduce individual risk of parasitism, since the best alternative, namely 'shielding in a herd' does not seem a successful strategy.

We consider the sensitivity of the model predictions to the incremental constant (*b*) in association with patch leaving decision rules and the Marginal Value Theorem (Charnov 1976). This theorem predicts that individual searching times in a patch are affected by the preceding travel time towards that patch, i.e., the distances between substrates, and average profitability across all patches (Charnov 1976, Godfray 1994). Increased travel time and reduced average profitability could prolong the optimal time spent in patch. In our model, we focussed only at single substrates and ignored all spatial characteristics. If the model would be expanded to a multi-substrate setting, travel times and average profitability could invoke significant effects on risk of parasitism when they act on the incremental constant (*b*), but to a far lesser extent when they act on initial motivation (M_0), since sensitivity to the latter parameter was relatively low. Similarly, the increased initial motivation (GUT) in response to the concentration of larval kairomones has only limited effects on the risk of parasitism, especially at high larval densities, where it is rapidly dominated by the increments.

The strong sensitivity of the model to the density of parasitoids, d_p, and the match in numbers of parasitoids and numbers of fly ovipositions, *m*, indicates that the pay-offs for individual parasitoids vary as a result of the behaviours and decisions of competing parasitoids. The optimal strategy for individual parasitoids is highly dependent on densities and characteristics of other parasitoids, and a game theoretical approach might be needed to appraise which strategies are optimal under various conditions (e.g., spatio-temporal distributions of host habitats, densities and characteristics of competing parasitoids). Until now, we focussed on the consequences of parasitoid behaviour for hosts and largely ignored the evolution of parasitoid behaviour itself. The strategies that parasitoids use have evolved under natural selection. Firstly and foremost, it should be remembered that the default values on numerical responses for our model are based on field observations, which do in fact depict the product of evolution. Selection will have driven parasitoid behaviour to choose those habitats and adjust searching times where host density, competitor density and individual decisions yield the highest profits in fitness terms. The currency that should be optimized for time-limited parasitoids is the rate of offspring gain (Stephens and Krebs 1986), but when a parasitoid encounters a substrate with high host densities (e.g., 250 larvae), she might become egg-limited instead. Such a lucky strike may be relatively rare (Janssen 1989), but the large fitness gain that can be achieved when anticipating such a lucky strike may select for an egg load that prevents egg-limitation (Ellers et al. 2000). We presented the model predictions for numbers of host attacked per wasp (n_a) (table 3, figs. 3, 4). These predicted values always increased monotonically with host density, and were sensitive to many parameters as well. When n_a was divided by total residence time ($Tt = T_s(n_a) + n_a \cdot t_h$) to describe the rate of offspring gain, these individual gains for parasitoids increased monotonically with each oviposition (fig. 6). Such a pattern likely reflects adaptation to clustered hosts. The increasing but decelerating rate of fitness gain could promote an initial arrestment-response of the parasitoid on a host-infested substrate, and subsequent adjustment of optimal patch residence time to travel time and across-habitat profitability (Charnov 1976).

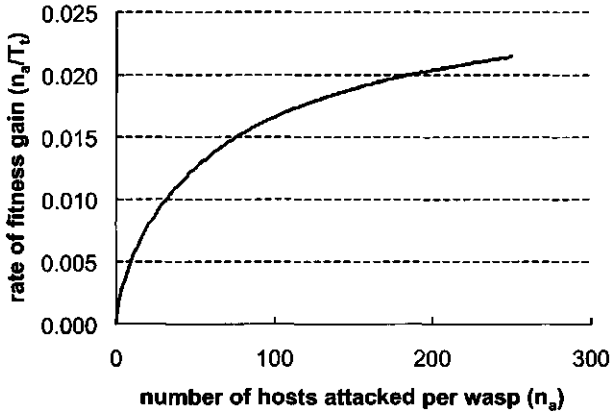

Figure 6: The predicted rate of offspring gain for parasitoids with an incremental mechanism of ovipositions on individual searching time.

With the inclusion of superparasitism through a reduction in patch depletion, we found essentially no effect on the risk of parasitism. Large influences on risk of parasitism are perhaps not to be expected, since the acceptance of parasitized hosts does not directly contribute to an increased risk for the hosts, but only indirectly, through increased individual searching times. Furthermore, superparasitism is not expected to be adopted immediately, but after a while (Visser et al. 1990), when increments are already relatively small, which even lessens the potential effects on searching time.

Alternative models and model components

A related model, The Random Parasite Equation (RPE) (Rogers 1972) also assumes random search within patches and measures over individual searching time. In theory, the Random Parasite Equation should have given equal predictions for the number of host attacked (N_a) as our model, since both models only substitute total residence time (T_r) in Nicholson's competition curve (eqn. 1a,b) by an estimation of individual searching time (T_s). The models, however, give dissimilarities in their predictions, and this is caused by an incorrect estimation of individual searching time in the RPE. The RPE assumes that upon encounters, parasitoids spend equal time handling parasitized and unparasitized larvae, and this total handling time is subtracted from the residence time ($T_s = T_r - N_e \cdot t_h$). *Leptopilina heterotoma*, however, and presumably most other parasitoids, can distinguish quickly between parasitized and unparasitized larvae and wastes only little time during encounters with already parasitized larvae (i.e., in rejections), unless she decides to superparasitize (Salt 1961, van Lenteren 1981). When using the RPE, the estimated total handling time that is subtracted from the measured residence time is erroneously large and therefore searching efficiency is artificially dampened. A model by Arditi (1983) differentiates between handling times of parasitized and unparasitized hosts. However, 'handling time' is only important with a fixed time horizon in mind, and becomes somewhat superfluous when parasitoids have a more flexible time horizon. The handling time as a prominent feature in type II functional responses and in the Random Parasite

Equation, is only applicable in situations where residence time is somehow fixed or constrained or when handling time is very long relative to average life time or searching time. For more flexible and perhaps biologically more realistic patch residence times, as arises from patch leaving rules with incremental mechanisms of ovipositions on searching time or motivation, the handling time itself does not necessarily play an appreciable role.

The original incremental mechanism of Waage can effectuate equal rates of fitness gains for parasitoids at patch leaving for patches with different numbers of hosts (Waage 1979, Wajnberg et al. 2000), which is in accordance with the predictions of the Marginal Value Theorem (Charnov 1976). In our data, the increments did vary between ovipositions as in Waage's model, only not with time between ovipositions, but with the numbers of previous ovipositions. We did not find the (counter-intuitive) positive relationship for the 'time between ovipositions' and the increment size. We assume that the only quantity that parasitoids keep track of is the number of hosts they parasitized. Consequently, the fitness gain curve in our model is the same at each host density. This mechanism, however, might give rise to a negative correlation between inter-oviposition times and motivation, as was found by Haccou et al. (1991). Depletion of unparasitized hosts in a substrate results in reduced oviposition rates and coincides with the diminishing increment sizes, but this relation is not causal.

Our choice for the incremental function, $I = \Sigma b/i$, was arbitrary, and based more on descriptive value than mechanistic insights. This function was based on data for relatively low densities of hosts, and this holds the risk that at higher larval densities, the function fails to accurately describe individual searching times of parasitoids. Observations on *L. boulardi* showed very similar 'diminishing return' patterns for residence times, that monotonically increased up till much higher larval densities (Hertlein and Thorarinsson 1987). This is no guarantee, but at least a re-assurance that our proposed incremental mechanism captures the right pattern for the full range of modelled host densities.

Population dynamics

Whereas we studied the influences of density dependence at the individual level (namely behaviour of searching parasitoids and individual risk for hosts), aggregation in parasitoid-host interactions is mostly studied in a population dynamic context. Parasitoid aggregation is renowned for its potential stabilizing effects at the population level (e.g., Hassell and May 1974, May 1978, Chesson and Murdoch 1986, Pacala et al. 1990, Ives 1992, Jaenike 1996). The effectiveness in stabilizing otherwise unstable population dynamics relies mainly on increasing the heterogeneity in the risk of parasitism across hosts (Chesson and Murdoch 1986, Ives 1992). A marked difference in patch residence time on substrates with high and low host densities was predicted to increase stability (Hassell and May 1974). Conversely, when optimal foraging or redistribution models were applied, density dependent aggregation by parasitoids only contributed to stability when sufficient heterogeneity was preserved, which depends among others on parasitoid density, parasitoid mobility and availability of searching time (reviewed by Hassell 2000). On the basis of our results, we speculate that it is doubtful whether the behavioural responses of *Leptopilina* in the *Drosophila* system would increase stability, because the combined numerical and functional responses homogenized the risk across a range of host densities.

Conclusion

We did not find dilution of risk at high host densities, but instead an ever increasing risk of parasitism. This was not caused by the numerical response, but by the flexible time allocation of the parasitoids. The numerical response towards the aggregation pheromone did augment the risk across *all* host densities. Therefore, the use of aggregation pheromones constitutes an ecological cost with respect to risk of parasitism, while the benefits of aggregative behaviour itself have to be sought in other directions than predator avoidance.

Acknowledgements

The students of the course Evolutionary Ecology in 1995 - 1999 are gratefully acknowledged for collecting the functional response data. We thank Lia Hemerik, Louise Vet and Marcel Dicke for valuable discussion and comments on the manuscript. Additional computer power for the simulations was made available by Pauline and Hugo Wertheim, Erik Schaberg and Tim Timmerman.

APPENDIX: DERIVATION OF THE EXPECTED NUMBER OF ATTACKED HOSTS

In this appendix, we justify the two assumptions that are implicit to the approximation procedure that we use to solve the two equations with two unknown quantities (2a or 2b and 3e). We do this by first giving an exact derivation for the expected number of attacked hosts at different host densities (N_t), and then showing that this is almost identical to the approximation that we use in the main text.

Exact derivation of n_a

Assume that one parasitoid (P = 1) searches on a patch with N_t hosts. The expected number of attacked hosts is denoted by $\langle n_a \rangle$ and can be calculated from

$$\langle n_a \rangle = \sum_{n_a=0}^{N_t} n_a \cdot p(n_a) \qquad \text{(A 1)}$$

where $p(n_a)$ is the probability for the parasitoid to find n_a hosts within the searching time T_s. Hence, we need to know $p(n_a)$ to calculate the expected number $\langle n_a \rangle$. This probability $p(n_a)$ depends on the number of encounters (N_e) of the parasitoid with hosts. N_e in turn depends on the searching time T_s of the parasitoid, which again depends on n_a. Rewriting these conditional probabilities in formula gives the following implicit equation for $p(n_a)$:

$$p(n_a) = \sum_{n_a'=0}^{N_t} \sum_{N_e=0}^{\max(N_e)} p(n_a \mid N_e) \cdot p(N_e \mid T_s(n_a')) \cdot p(n_a') \qquad \text{(A 2)}$$

where the prime is used in n_a' only to distinguish it in the summation from n_a. To solve (A 2) for $p(n_a)$, we need to know the ingredients $T_s(n_a')$, $\max(N_e)$, $p(N_e \mid T_s(n_a'))$ and $p(n_a \mid N_e)$. We derive expressions for these ingredients in order.

- $T_s(n_a')$

We assume (see main text) that searching time T_s is described by

$$T_s(n_a') = M_0 + \sum_{i=1}^{n_a'} \frac{b}{i} \qquad \text{(A 3)}$$

where M_0 is the initial motivation and b an incremental constant.

- $\max(N_e)$

The parasitoid searches for hosts by probing randomly in the substrate, with a constant frequency (pf). The maximum number of probes in a period of duration $T_s(n_a')$ sets the maximum to the number of encounters:

$$\max(N_e) = T_s(n_a') \cdot pf \qquad \text{(A 4)}$$

To allow for summation from zero until $\max(N_e)$, we assume for the moment that $\max(N_e)$ is an integer.

- $p(N_e \mid T_s(n_a'))$

The probability per probe that a host is hit with the ovipositor equals $(N_t \cdot A_l / A_p)$ where A_l and A_p are the surface areas of a host and the patch respectively. The probability per probe of a failure is $(1 - N_t \cdot A_l / A_p)$. The probability of N_e encounters, given a search time of $T_s(n_a')$, is then binomially distributed:

$$p(N_e \mid T_s(n_a')) = \binom{T_s(n_a') \cdot pf}{N_e} \cdot \left(\frac{N_t \cdot A_l}{A_p}\right)^{N_e} \cdot \left(1 - \frac{N_t \cdot A_l}{A_p}\right)^{T_s(n_a') \cdot pf - N_e} \qquad \text{(A 5)}$$

where $\binom{T_s(n_a') \cdot pf}{N_e}$ is a combinatorial, defined as $\binom{x}{y} = \frac{x!}{y! \cdot (x - y)!}$

that describes the number of possible sequences to draw y hits out of x probes.

- $p(n_a \mid N_e)$

For each encounter, N_t hosts are available, so for N_e encounters, we have N_t^{Ne} possible combinations for these N_e encounters. Let us denote $Mn_a(N_e)$ as the number of possibilities that N_e encounters are with a specific combination of n_a hosts. The number of specific combinations of n_a hosts out of a total number of N_t hosts equals $\binom{N_t}{n_a}$. The product of $Mn_a(N_e)$ and $\binom{N_t}{n_a}$ describes the number of

possibilities that N_e encounters are with precisely n_a hosts. This product divided by all possible combinations describes the probability of n_a attacked hosts, given N_e encounters:

$$p(n_a|N_e) = \binom{N_t}{n_a} \cdot \frac{M_{n_a}}{N_t^{N_e}} \tag{A 6}$$

If encounters are restricted to n_a hosts, and for each encounter n_a larvae are available, we have n_a^{Ne} possible combinations for N_e encounters. This number also includes the possibilities that less than n_a hosts are encountered. Hence, we need to subtract these possibilities from the n_a^{Ne} possible combinations:

$$M_{n_a}(N_e) = n_a^{N_e} - \sum_{j=0}^{n_a-1} \binom{n_a}{j} \cdot M_j(N_e) \tag{A 7a}$$

which is a recurrent relationship for $M_{na}(N_e)$. This gives

$$M_{n_a}(N_e) = \sum_{j=0}^{n_a} \binom{n_a}{j} \cdot j^{N_e} \cdot (-1)^{n_a+j} \tag{A 7b}$$

This can be proved by showing that

$$n_a^{(N_e)} - \sum_{j=0}^{n_a-1} \binom{n_a}{j} \cdot \sum_{i=0}^{j} \binom{j}{i} \cdot (-1)^{j+i} \cdot i^{N_e} = \sum_{j=0}^{n_a} \binom{n_a}{j} \cdot j^{N_e} \cdot (-1)^{n_a+j} \tag{A 7c}$$

which is straightforward after changing the order of summation in the left hand side of (A 7c).

Having found all ingredients of (A 2), we can write

$$p(n_a) = \sum_{n_a'=0}^{N_t} \sum_{N_e=0}^{\max(N_e)} (p(n_a|N_e) \cdot p(N_e|T_s(n_a')) \cdot p(n_a') =$$

$$= \sum_{n_a'=0}^{N_t} \sum_{N_e=0}^{T_s(n_a')\cdot pf} \sum_{j=0}^{n_a} \binom{N_t}{n_a} \cdot \binom{n_a}{j} \cdot \left(\frac{j}{N_t}\right)^{N_e} \cdot (-1)^{n_a+j} \cdot \tag{A 8a}$$

$$\cdot \binom{T_s(n_a')\cdot pf}{N_e} \cdot \left(\frac{N_t \cdot A_l}{A_p}\right)^{N_e} \cdot \left(1 - \frac{N_t \cdot A_l}{A_p}\right)^{T_s(n_a')\cdot pf - N_e} \cdot p(n_a') =$$

using Newton's binomium formula: $(p+q)^n = \binom{n}{i} \cdot p^i \cdot q^{n-i}$

$$= \sum_{n_a'=0}^{N_t} \sum_{j=0}^{n_a} \binom{N_t}{n_a} \cdot \binom{n_a}{j} \cdot (-1)^{n_a+j} \cdot p(n_a') \cdot \tag{A 8b}$$

$$\cdot \sum_{N_e=0}^{T_s(n_a')\cdot pf} \binom{T_s(n_a')\cdot pf}{N_e} \cdot \left(\frac{A_l \cdot j}{A_p}\right)^{N_e} \cdot \left(1 - \frac{N_t \cdot A_l}{A_p}\right)^{T_s(n_a')\cdot pf - N_e} =$$

$$= \sum_{n_a'=0}^{N_t} \left[\sum_{j=0}^{n_a} \binom{N_t}{n_a} \cdot \binom{n_a}{j} \cdot (-1)^{n_a+j} \cdot \left(1 - \frac{A_l}{A_p} \cdot (N_t - j) \right)^{T_s(n_a') \cdot pf} \right] \cdot p(n_a') = \quad \text{(A 8c)}$$

$$= \sum_{n_a=0}^{N_t} \boldsymbol{B}(n_a, n_a') \cdot p(n_a') \Rightarrow \quad \text{(A 8d)}$$

$$\vec{p}(n_a) = \boldsymbol{B} \cdot \vec{p}(n_a') \quad \text{(A 8e)}$$

with $\vec{p}$ a vector of probabilities $p(n_a)$, $\vec{p} = \begin{pmatrix} p(0) \\ p(1) \\ \vdots \\ p(N_t) \end{pmatrix}$ and **B** a matrix with elements (A 8f)

$$\boldsymbol{B}(n_a, n_a') = \sum_{j=0}^{n_a} \binom{N_t}{n_a} \cdot \binom{n_a}{j} \cdot (-1)^{n_a+j} \cdot \left(1 - \frac{A_l}{A_p} \cdot (N_t - j) \right)^{T_s(n_a') \cdot pf} \quad \text{(A 8g)}$$

which can be calculated numerically. If we substitute this solution for $p(n_a)$ into equation (A 1), we arrive at the expected number of attacked hosts $\langle n_a \rangle$. Equation (A 8e) is in fact an eigenvalue equation for **B** for the eigenvalue of 1. Therefore, the solution to this equation $\vec{p}$ is the eigenvector corresponding to the eigenvalue 1, normalized such that $\sum_{n_a=0}^{N_t} p(n_a) = 1$. This can be computed numerically, but becomes increasingly strenuous for larger values of N_t. The expression is no longer restricted to integer values of $T_s(n_a') \cdot pf$, although for exactness, we should round $T_s(n_a')$ down to the nearest integer.

We have assumed so far that only one parasitoid searches on the patch. If there are P parasitoids, we can act as if there is only one, but with a value of $\max(N_e) = P \cdot T_s(n_a) \cdot pf$.

Approximation procedure

In the main text, we use a much simpler approximation to obtain $\langle n_a \rangle$. We will now derive this approximation, again for $P = 1$, which can be easily extended to other values of P as before. We first form an expression for the conditional expectation $\langle n_a \mid N_e \rangle$. For each encounter, all hosts have a proportionate chance $(1 / N_t)$ of being encountered. Thus, the probability for a host to avoid all N_e encounters is $(1 - 1 / N_t)^{Ne}$ and the probability that it will be encountered at least once equals $1 - (1 - 1 / N_t)^{Ne}$. For N_t hosts, the expected number of attacked hosts is therefore

$$\langle n_a | N_e \rangle = N_t \cdot \left[1 - \left(1 - \frac{1}{N_t} \right)^{N_e} \right] \quad \text{(A 9)}$$

We combine equations (A 1) and (A 2) to derive

$$\langle n_a \rangle = \sum_{n_a=0}^{N_t} n_a \cdot \sum_{n_a'=0}^{N_t} \sum_{N_e=0}^{\max(N_e)} p(n_a \mid N_e) \cdot p(N_e \mid T_s(n_a')) \cdot p(n_a') = \quad \text{(A 10a)}$$

$$= \sum_{n_a'=0}^{N_t} \sum_{N_e=0}^{T_s(n_a')\cdot pf} \left(\sum_{n_a}^{N_t} n_a \cdot p(n_a|N_e) \right) \cdot p(N_e \mid T_s(n_a')) \cdot p(n_a') = \quad \text{(A 10b)}$$

$$= \sum_{n_a'=0}^{N_t} \sum_{N_e=0}^{T_s(n_a')\cdot pf} \langle n_a \mid N_e \rangle \cdot p(N_e \mid T_s(n_a')) \cdot p(n_a') = \quad \text{(A 10c)}$$

$$= \left\langle N_t \cdot \left[1 - \left(1 - \frac{A_l}{A_p} \right)^{T_s(n_a')\cdot pf} \right] \right\rangle \quad \text{(A 10d)}$$

where we have applied the definition of expectation (A 1) to the term in brackets in (A 10b) to obtain (A 10c), we have substituted the expressions for $\langle n_a \mid N_e \rangle$ and $p(N_e \mid T_s(n_a'))$, we grouped all similar exponents and used the Newton binomium formula, and we have again applied the definition of expectation to get the final result (A 10d).

Now, recall that encounter rate, a', is defined by $a' = \frac{A_l}{A_p} \cdot pf$ (A 11)

If we assume 1) that the expectation of a function of x is the function of the expectation of x (which is not true in general for non-linear relationships!) and 2) that A_p / A_l is large ($\gg 1$), then we have

$$\langle n_a \rangle = \left\langle N_t \cdot \left[1 - \left(1 - \frac{A_l}{A_p} \right)^{T_s(n_a')\cdot pf} \right] \right\rangle = \quad \text{(A 12a)}$$

$$= \left\langle N_t \cdot \left[1 - \left(1 - \frac{1}{\frac{A_p}{A_l}} \right)^{a' \cdot T_s(n_a') \cdot \frac{A_p}{A_l}} \right] \right\rangle \approx \quad \text{(A 12b)}$$

$$\approx N_t \cdot \left[1 - \left(1 - \frac{1}{\frac{A_p}{A_l}} \right)^{a' \cdot T_s(\langle n_a' \rangle) \cdot \frac{A_p}{A_l}} \right] \approx \quad \text{(A 12c)}$$

$$= N_t \cdot \left[1 - \left\{ \left(1 - \frac{1}{\frac{A_p}{A_l}} \right)^{\frac{A_p}{A_l}} \right\}^{a' \cdot T_s(\langle n_a' \rangle)} \right] \approx \quad \text{(A 12d)}$$

using $(1 + \frac{1}{Z})^Z \approx e$,

$$\approx N_t \cdot \left[1 - e^{-a' \cdot T_s(\langle n_a' \rangle)} \right] \quad \text{(A 12e)}$$

With this equation (A 12e) we have a simple implicit expression for $\langle n_a \rangle$ which can be easily solved numerically for any N_t, in contrast to the calculation of the eigenvector of **B** corresponding to the eigenvalue 1 which we had previously. To be able to calculate $T_s(\langle n_a \rangle)$ for non-integer values of $\langle n_a \rangle$, we note that

$$T_s(n_a) = M_0 + \sum_{i=1}^{n_a} \frac{b}{i} = M_0 + b \cdot (\psi(n_a + 1) + \gamma) \qquad \text{(A 13)}$$

where $\psi(z)$ is the digamma function defined for any real (even complex) argument z and γ is Euler's gamma constant (≈ 0.57722).

To judge how well our approximation works, we have plotted the quotient of the approximate and exact solutions for $\langle n_a \rangle$ as a function of N_t in fig. A 1.

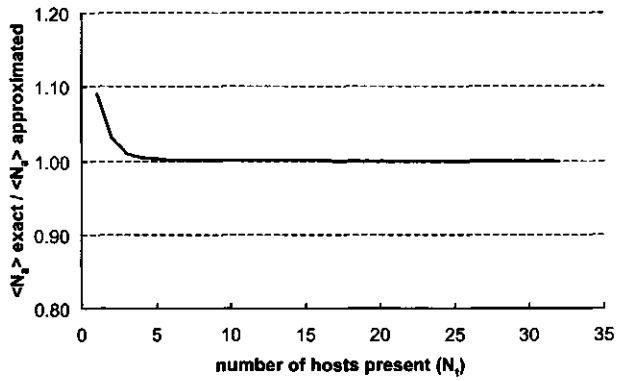

Figure A–1: The predicted values for N_a, as calculated with the exact and the approximation procedure. A ratio of unity indicates that the approximation procedure yields the same predicted values as the exact procedure.

CHAPTER 8

The interaction between dispersal, the Allee effect and scramble competition affects population dynamics: a case study on *Drosophila*

Abstract

Many organisms experience an Allee effect: population growth is hampered at low densities. In addition, individuals compete with one another at high densities. The Allee effect and competition thus create a lower and an upper bound to local population size. Local populations can, however, be connected through dispersal. By using a spatio-temporal simulation model, parameterised for *Drosophila melanogaster*, we explore the consequences of the Allee effect, scramble competition and dispersal for different combinations of resource distributions, initial adult distributions, modes of dispersal and boundary conditions.

We found that the initial distribution of adults determines whether a population can establish, while resource availability, the ability to reach resources and heterogeneity are mainly responsible for subsequent population persistence. In our model heterogeneity was introduced by the distribution of resources, the initial adult distribution, and the boundary conditions. Although local population dynamics are inherently unstable, overall stability can be attained by (re)colonisation processes. The averaged dynamics of the total population turned out to be reasonably smooth, so apparently upper and lower local population bounds, coupled with dispersal, created an effectively stable mean population size for the system as a whole. This suggests that stable mean population sizes for spatial populations can be emergent properties appearing at sufficiently large scales, as opposed to inherent properties occurring at all scales.

We also found, in agreement with most literature but contrary to some recent literature, that population persistence can be facilitated by a leptokurtic dispersal mode, which has higher probabilities of travelling both short and long distances, but smaller probability of travelling intermediate distances than random dispersal.

Introduction

Two basic ecological mechanisms set lower and upper bounds to the size of local populations: the Allee effect and competition (Begon et al. 1996). The Allee effect is defined as a reduced population growth rate at low densities. This effect can be caused by difficulties in, for example, mate finding, food exploitation (e.g. host resistance can only be overcome by sufficient numbers of consumers) and predator avoidance or defence (Allee 1931, Courchamp et al. 1999, Stephens and Sutherland 1999, Stephens et al. 1999). Naturally occurring aggregated distributions of organisms over the available resources may indicate the presence of an Allee effect, because the Allee effect might select for aggregation behaviour (Stephens and Sutherland 1999). Such behaviour can, for example, be induced by individuals excreting substances to attract conspecifics (aggregation pheromones, Shorey 1973).

At high local population densities competition may lead to lower population growth rate as well. Competition for resources (food, space and mates) ranges between contest competition and scramble competition. With contest competition a limited number of individuals obtain a sufficient share of resource while the excess individuals get nothing at all, whereas with scramble competition all individuals obtain an equal share of the resource which may or may not be sufficient (Calow et al. 1998).

A third fundamental ecological process, dispersal, couples the dynamics within and between populations. We regard dispersal as any kind of spatial displacement, not just restricted to a metapopulation setting. It has long been recognised that dispersal enables individuals to leave unfavourable habitat, avoid predation or competition, find new ephemeral resources, search for mates, evade inbreeding, and (re)colonise areas (for a review, see Begon et al. 1996). Whether they are successful in doing so depends on the interplay between dispersal and other ecological mechanisms.

In this paper we will study how the interaction between dispersal, the Allee effect and scramble competition – setting lower and upper bounds to population size – determines the establishment and persistence of a population. By establishment we mean the production of a second generation, and by persistence the production of several (20) generations.

We make two assumptions. Firstly, we assume that resources are exhaustible within the time scale of scramble competition. Secondly, we assume that population growth rate is positive as long as resources are available. This entails unstable local population dynamics (see also Etienne et al. 2000). Dispersal may stabilise the dynamics at a larger spatial scale, but might be counteracted by the Allee effect. The precise balance of the above-mentioned processes is not obvious at face value. In the metapopulation literature some attention has been paid to similar processes (Amarasekare 1998, Doebeli and Ruxton 1998, Gyllenberg et al. 1999). However, similar mechanisms may be at work in populations without a (classical) metapopulation structure. For such situations some theoretical work exists in one spatial dimension (Kot et al. 1996, Lewis and Kareiva 1993). In this paper, we will formulate a simple model in two spatial dimensions that captures the essence of the interaction between dispersal, Allee effect and scramble competition. This model is a caricature of real populations, but resembles the population biology of fruit flies. We use the population biology of the frugivorous *Drosophila melanogaster* to illustrate the combined effects of these three processes.

Drosophilid fruit flies have aggregated distributions (Rosewell et al. 1990, Sevenster and van Alphen 1996, Wertheim et al. 2000, produce aggregation pheromones (Bartelt et al. 1985b) and may be assumed to experience an Allee effect (Stephens and Sutherland 1999). In addition, they compete for limited food resources (Bakker 1961, Grimaldi and Jaenike 1984, Sevenster 1992). They breed in ephemeral and patchy substrates (fermenting fruit and sap streams, rotting plant material and mushrooms). After one generation resources are generally completely exhausted and newly eclosed adults must disperse to find new breeding sites. Inseminated females distribute their eggs in clutches over several resources over a period of several days, and along with the eggs deposit micro-organisms like bacteria and yeast (Gilbert 1980). The eggs hatch and emerging larvae are confined to a single resource. Fermenting yeasts develop on the resource and constitute the main larval food source. At low larval densities an Allee effect may be active. We assume that the Allee effect is caused by difficulties in larval resource exploitation. Two possible underlying mechanisms are: adult flies in low numbers are incapable of introducing sufficient numbers of micro-organisms to render a substrate into a fermenting resource, or low larval densities amount to little tunnelling and defecation thereby slowing down the fermentation process, and may therefore be insufficient to cultivate the resource (see also chapter 5). At high densities the larvae often experience severe competition for food. This competition is predominantly of the scramble type. This means that either almost all individuals of a cohort (simultaneously eclosed larvae) will be able to complete their development or almost none (Bakker 1961, Sang 1956). Thus, within a resource item the Allee effect and scramble competition set lower and upper bounds to the number of eggs below and above which only very few larvae can successfully develop.

In summary, with a spatio-temporal simulation model loosely based on *Drosophila* we will investigate the combined effects of dispersal, Allee effect and scramble competition on establishment and persistence of populations that have inherently unstable local dynamics. The model we use for population dynamics is as simple as possible (deterministic, linear growth with lower and upper lethal limits for the Allee effect and scramble competition, respectively), but it portrays the effect of finite, ephemeral resources which require a critical population size to exploit. We explore the effects for different combinations of resource distributions (which in one case we allow to be stochastic), initial adult distributions, initial adult densities, modes of dispersal and boundary conditions.

Outline of the modelling procedure

We used an integrodifference approach as in Neubert et al. (1995). In this approach time is discrete and space is continuous, and growth and dispersal are distinct phases. This allows us to model adult dispersal and larval competition separately. We modelled a fruit fly population in a two-dimensional environment in 20 discrete generations, each of which consists of three steps (e.g. days) of dispersal by adult females and one larval development step. After each dispersal step only those (female) adults which are on resource items deposit f eggs. The cumulative number of eggs on each resource item after three dispersal steps determines whether larvae will develop successfully: all larvae in one substrate survive when their

number lies between the lower bound L^{min} set by the Allee effect and the upper bound L^{max} set by scramble competition; otherwise, all larvae die. We assumed that the surviving larvae have a 1:1 sex ratio; therefore, half of them constitute the next female adult population. The outcome of the Allee effect and competition in the larval development phase is expressed in the following formula:

$$A_{n+1}(x,y)=\frac{1}{2}\begin{cases}0 & L_{n+1}(x,y)<L^{\min}\\ L_{n+1}(x,y) & L^{\min}\le L_{n+1}(x,y)\le L^{\max}\\ 0 & L_{n+1}(x,y)>L^{\max}\end{cases}$$

The terms to the right of the bracket describe the local dynamics in each spatial coordinate (x, y) and set the conditions for the survival of larvae, molting into the next generation of dispersing females.

Dispersal and oviposition can be summarised in the following formula:

$$L_{n+1} = f(R)\,K * f(R)\,K * f(R)\,K * A_n,$$

where $f(R)$ is the fecundity which is equal to f if $R > 0$ and equal to 0 otherwise, because oviposition only occurs on resources, A_n is the female adult population in generation n at the beginning of the dispersal phase. L_{n+1} is the larval population in generation $n + 1$, K describes the dispersal pattern (see below) and * denotes the convolution operator as in Allen et al. (1996) and Brewster et al. (1999). The convolution operator is short notation for a summation over all spatial coordinates, i.e.:

$$(p * q)(x,y) := \iint p(x-x', y-y')q(x', y')\mathrm{d}x'\mathrm{d}y'.$$

Three convolutions are used to model the spatial distribution of eggs by females over a series of three days; each term $f\,K$ * corresponds to a day of dispersal and oviposition. The daily fecundity was set at $f = 7$, corresponding to an average of 7 viable eggs per female, as found in field data (Boulétreau 1978).

Dispersal

We take dispersal to be described by one of three dispersal kernels K: (K_R) random, (K_{DE}) double exponential and (K_{RR}) ring random dispersal.

Random dispersal, or diffusion, is described by

$$K_R(x,y)=\frac{1}{4\pi D}e^{-\frac{x^2+y^2}{4D}}$$

where D is the so-called diffusion constant which measures the area traversed per unit of time, and hence reflects the speed of movement. The expected displacement for random dispersal is given by $\sigma\sqrt{(\pi/2)}$ where $\sigma^2 = 2D$. Timofeef-Ressovsky and Timofeef-Ressovsky (1940) report that *D. melanogaster* moves

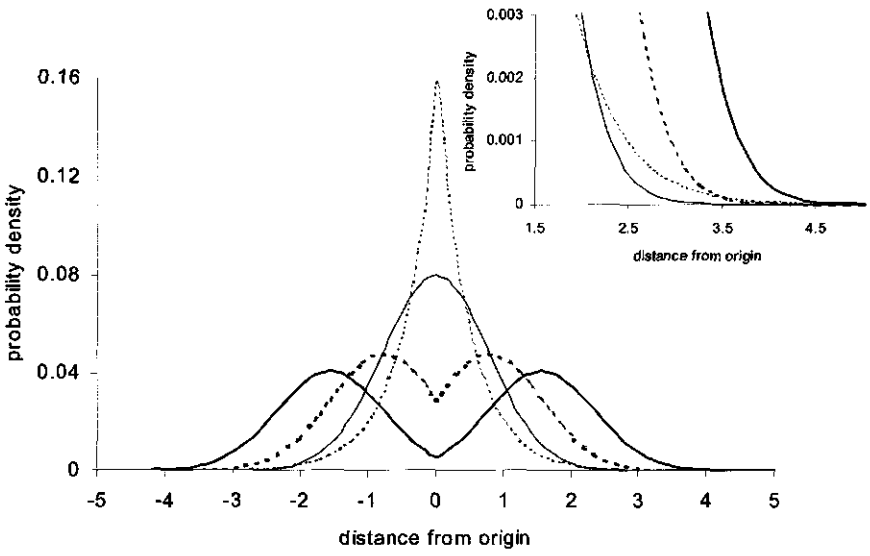

Figure 1: The projection of the probability density functions of three dispersal kernels on a vertical plane through the origin: random (solid line), double exponential (dotted line), ring random with $\rho = 5$ m (thick dotted line) and $\rho = 10$ m (thick solid line). The expected displacements of the random and the double exponential kernel are equal. The inset shows that the tail of the double exponential is fatter than that of the other kernels.

10 m or less per day in the field. We chose σ equal to half this distance (i.e. $\sigma = 5$ m) and thus $D = 12.5$ m^{2}day^{-1}.

Double-exponential dispersal, as proposed by Neubert et al. (1995) and Kot et al. (1996), assumes random motion *and* settlement at a constant rate α. It is described by

$$K_{DE}(x,y) = \frac{\lambda^2}{2\pi} e^{-\lambda\sqrt{x^2+y^2}}$$

where λ is a function of D and the settlement rate α. To fairly compare the random and the double exponential dispersal kernels we imposed the condition that the expected displacements are equal. This implies that

$$\lambda = \frac{2\sqrt{2}}{\sigma\sqrt{\pi}}$$

Ring random dispersal, described by

$$K_{RR}(x,y) = \frac{N}{4\pi D} e^{-\frac{(\sqrt{x^2+y^2}-\rho)^2}{4D}}$$

where N is a normalisation constant, represents adults first moving away a distance ρ and then distributing randomly. We treated the ring random dispersal kernel as a perturbation of the random dispersal kernel in order to evaluate the sensitivity of the model to deviations from the random dispersal kernel. Therefore we took ρ of the order of σ, namely $\rho = 5$ m and 10 m. For these parameter settings the kernels are plotted in figure 1.

Upper and lower bounds

We used apples as the model resource for larval development. One apple can support development of at most 200 to 300 *D. melanogaster* larvae (Spencer (1950), Sang (1956)). We set our upper bound at $L^{max} = 250$ larvae. For the lower bound no specific estimate is available in the literature, but high mortality is found at low larval densities by Sang (1956) (8 larvae: 40%; 50 larvae: 15%). Therefore we (arbitrarily) chose $L^{min} = 25$ larvae per apple.

Boundary conditions

We defined a grid (128 m by 128 m) with three different boundary conditions: periodic, reflecting (no migration over the boundaries) and absorbing (lethal) boundaries. Periodic boundaries signify that fruit flies that leave the grid on the left (or upper) side, re-enter at the right (or lower) side and vice versa. At a reflecting boundary they bounce back, and at the absorbing boundary they disappear. Periodic boundaries make modelling easy, but are not entirely realistic. Using periodic boundaries is permissible for a closed system in which immigration and emigration cancel each other: for each *Drosophila* that leaves the simulated area a new one enters. Closed systems where no *Drosophila* can leave the area are described by reflecting boundary conditions. Absorbing boundary conditions represent an open system where *Drosophila* can leave the habitat but never (re-)enter it.

These boundary conditions were implemented using reflections of the spatially extended population data at each dispersal step. This is equivalent to using the method of images for free-space Green's functions (see Duchateau and Zachmann 1986) to create a dispersal kernel that accounts for the boundary conditions. For example, for lethal boundaries the data are reflected negatively across the boundary and then dispersal proceeds as usual. As long as the dispersal kernels involved are axisymmetric this methodology implements the boundaries transparently and without error.

Resource distribution

In the grid we placed resource items (apples). Each grid cell of 1 m by 1 m contained one resource item or none. Because persistence in a spatial environment may depend on the resource distribution, we used three different resource distributions representing different levels of heterogeneity: (1) *Spatially homogeneous*: Apples are present in every grid cell. (2) *Spatially structured*: Apples are present only in specified patches of cells in a 120 m by 120 m grid; this creates a border of 4 m on all sides. (3) *Spatio-temporally heterogeneous*: Apples are randomly distributed and redistributed over all grid cells in both space and time, modelling random windfall of apples. Note that only with resource distribution (2) our model could be considered as a metapopulation in the classical sense, that is, with several isolated fixed habitat patches.

In case (2) we chose patches of (A) 8 m by 8 m, with inter-patch distances of 0, 2, 4, 7, 12, 16, 22, 32 and 52 m, (B) 16 m by 16 m with inter-patch distances of 4, 8, 14, 24 and 44 m, (C) 24 m by 24 m with inter-patch distances of 6, 16 and 36 m, and (D) 32 m by 32 m with inter-patch distance 28 m spread out evenly over space. As the inter-patch distances decrease, the corresponding number of patches obviously increases. The total amount of resources may differ considerably between all these configurations. In case (3) we varied the number of cells $n = k^2$ in which we placed apples by varying k from 10 to 120 with steps of 10. After each generation apples were replaced in the configuration of the initial distribution for cases (1) and (2). In case (3) apples were randomly redistributed, which reflects temporal heterogeneity. This is the only place where we introduce stochasticity into our simulation model. Because of the randomness we ran 10 simulations in this case. Although a larger number of replicates would improve the reliability of the quantitative results, these simulations are very time-consuming, and the chosen number of replicates turned out to be sufficient to capture the qualitative pattern which is our main interest.

Initial adult distribution

Persistence in a spatial environment may also depend on the initial adult female distribution. From hereon we refer to the adult females simply as adults. We used three different initial adult distributions: (I) *Homogeneous*: Adults introduced in every grid cell. (II) *4-Cell source*: Adults introduced in four cells (2 m by 2 m) containing resources closest to the centre of the grid. (III) *256-Cell source*: Adults introduced in 256 cells (16 m by 16 m) containing resources closest to the center of the grid. In cases (II) and (III) we varied the initial number of adults in each cell, P_0, from half of the lower bound to half of the upper bound, i.e. between 12.5 and 125 with steps of 12.5. In case (I) the upper bound is almost always immediately exceeded with these values, because with a homogeneous initial adult distribution the total number of eggs deposited in *each* resource item is approximately P_0 times 21 eggs (3 days of 7 eggs per day). To circumvent this problem we varied P_0 in this case between 2.5 and 25 with steps of 2.5. Although this starting condition can never emerge from the simulation model itself and thus seems to contradict our assumptions, it can represent a more complex situation, such as *Drosophila* being already present in the environment (e.g. on other resources or emerging from diapause), and then switching to apples in low densities. In preliminary simulations we also considered a 1-cell source initial adult distribution, but this always fails to establish irrespective of the resource distribution because the total initial adult numbers are too low; the critical number of eggs to surpass the lower boundary is not reached in any of the resource items.

We recorded the total number of adults at each generation, and whether or not the population had established and persisted.

Results

The general conclusion from the simulations is: the initial distribution and density of adults determines whether a population can establish (i.e. produce a second generation), while resource availability and heterogeneity are mainly responsible for persistence (i.e. exist after 20 generations). Spatio-temporal heterogeneity can be introduced by the distribution of resources and of initial adults, and by the boundary conditions. In situations where the *D. melanogaster* population can establish and exist for at least several generations, most initial adult distributions lead to a qualitatively similar dynamical pattern of the total population size. Yet, population dynamics in each grid cell are highly variable and inherently unstable as can be seen in figure 2 and figure 3; overall stable population sizes can only be attained by local extinction and recolonisation processes.

Periodic boundary conditions and random dispersal

Populations fail to establish for a 4-cell source adult distribution (II) with low P_0 ($P_0 \leq 75$) and for a homogeneous adult distribution (I) with high P_0 ($P_0 \geq 10$), because these result in egg distributions outside the range (L^{min}, L^{max}) on all resource items, regardless of the resource distribution. For the 256-cell initial adult distribution (III) establishment is almost always achieved, except in a spatially structured environment (2) with inter-patch distances greater than or equal to 16 m. Population persistence depends on the resource distribution; therefore we will describe the results for each resource distribution separately.

Homogeneous resource distribution

Without dispersal any distribution of adults is doomed to extinction (Etienne *et al.* (2000)). With dispersal a homogeneous initial distribution of adults (I) is still doomed to extinction for all P_0 in a homogeneous resource distribution, because symmetric dispersal has no net effect in a purely homogeneous world. A small initial *local* population as in cases (II) and (III), however, can persist if initial numbers of adults are sufficiently large (II: $P_0 \geq 87.5$; III: all P_0). When the total number of adult fruit flies levels off (see figure 2

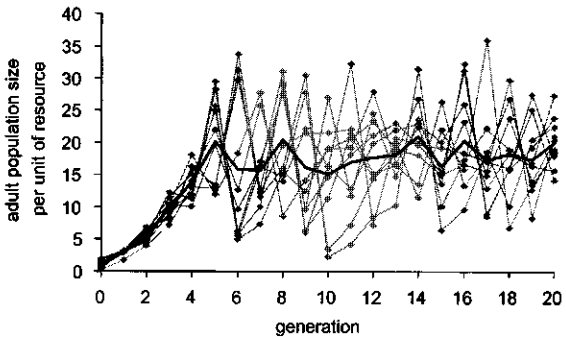

Figure 2: The development of the adult population size divided by the total number of resource items during 20 generations for different values of P_0 (grey lines) the average of which is the black line. Dispersal is random, the initial adult distribution is the 256-cell source (III) and the resource distribution is homogeneous (1).

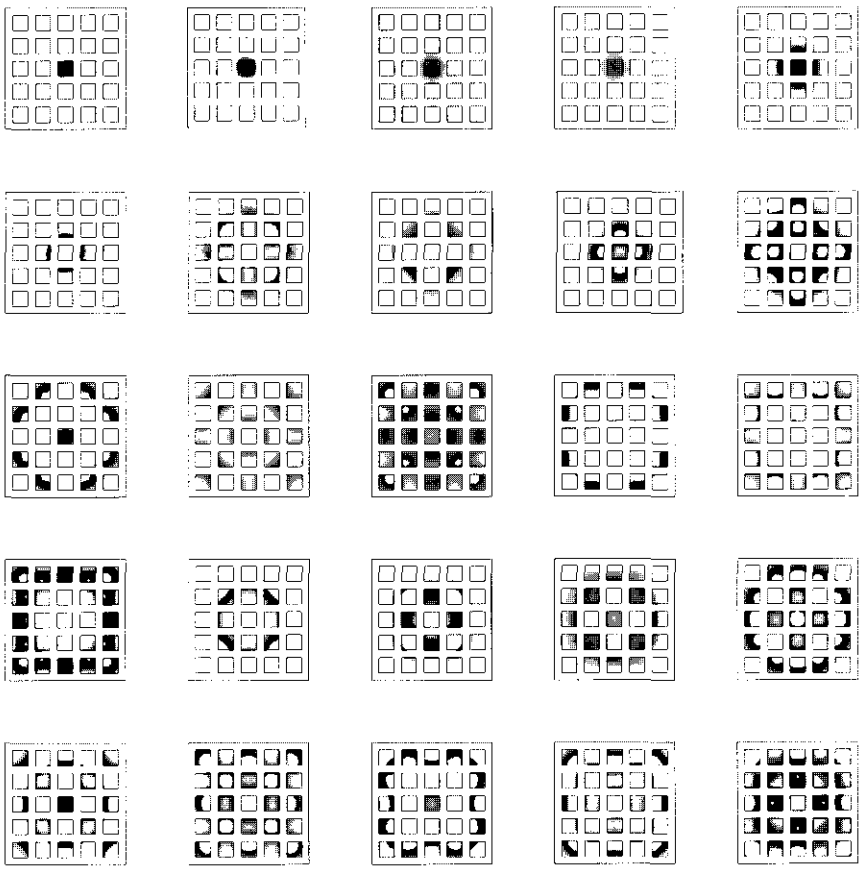

Figure 3: Snapshots of simulation results of the 256-cell source (III) in a spatially structured environment (2) of 16 m by 16 m patches containing apples at inter-patch distance of 8 m, and $P_0 = 125$. The darker shades denote higher adult fruit fly density. The first snapshot is the initial adult distribution (III). The next three snapshots are the three random dispersal steps directly following this initial distribution. The fifth snapshot shows the cumulative number of eggs after three dispersal steps. The sixth snapshot shows the resulting new generation of fruit flies that were able to overcome the Allee effect and scramble competition. The other snapshots are taken at the same point during the following generations: right after the fruit flies come out of the apples (i.e. right before dispersal).

for an example), the average number of adults per resource is about 20. Over grid cells the variation in adult number per resource item is large: many resources are not occupied and many are heavily overexploited.

Spatially structured resource distribution

For an example of population dynamics in a spatially structured environment with a certain initial adult distribution we refer to figure 3.

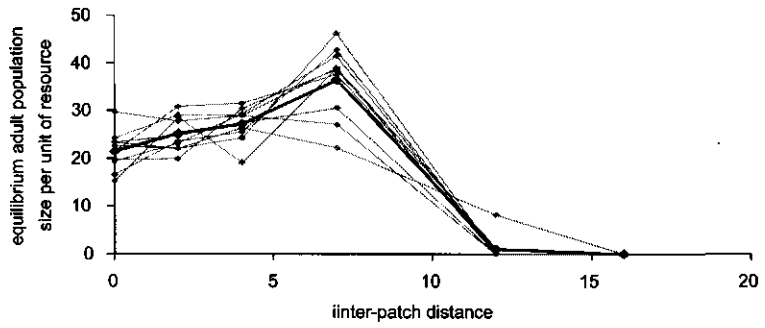

Figure 4: The equilibrium level of the adult population size per unit of resource (i.e. its value at generation 20, see figure 2) versus inter-patch distance for several values of P_0 (grey lines) the average of which is the black line. Dispersal is random, the initial adult distribution is the 256-cell source (III) and the resource distribution is spatially structured (2) with patches of 8 m by 8 m.

Whether populations persist depends on P_0 and on the interplay between patch size and inter-patch distance. Inter-patch distances of 12 m can be bridged, but from 16 m onwards, other islands of resource are not attainable in sufficient numbers using the dispersal parameters considered (see fig. 4). It also appeared that a resource patch of 32 m by 32 m is only just too small to sustain a stable population whereas the homogeneous grid was large enough. Only in a situation where patches are close or large enough can a long-term global persistence of populations be achieved. In our spatially structured simulations the patches were never sufficiently large to allow for persistence without connectedness to other patches.

Spatio-temporally heterogeneous resource distribution

We found that the *D. melanogaster* population can only persist in an environment with randomly distributed resources if the number of (cells containing) resource items is above a certain minimum which corresponds to a maximum average distance between resource items. For the homogeneous initial adult distribution (I) a stable total population size is possible only for low P_0 and a number of resource items between a minimum of 1600 and a maximum of 8100. This maximum arises because a high number of resource items resembles a homogeneous resource distribution, which leads to extinction (see above). Even for numbers of resource items between 1600 and 8100, populations sometimes did not persist due to overexploitation in early generations. For the 4-cell source initial adult distribution (II), persistence was possible when resources were available in 4900 cells or more, depending on P_0 (figure 5). For the 256-cell source (III), 2500 cells or more always yielded persistent populations regardless of the value of P_0.

Figure 5: The percentage of trials (from a total of 10) yielding persistence with the number of resource items $\geq 70^2$. The 5 bars at each value of the number of resource items correspond to 5 values of P_0 (a = 75, b = 87.5, c = 100, d = 112.5 and e = 125). For lower numbers of resource items and for lower P_0 persistence was never found. Dispersal is random, the initial adult distribution is the 4-cell source (II) and the resource distribution is spatio-temporally heterogeneous (3).

The equilibrium number of adults per resource item decreases with increasing number of cells containing resource items (i.e. decreasing inter-patch distances). The chance of reaching a resource item during dispersal then increases, and thus fewer adults are needed to achieve local densities above the critical lower bound. For the local initial adult distributions ((II) and (III)) the average population size per unit of resource turned out to be largely independent of P_0 (see figure 6).

Reflecting and absorbing boundary conditions

The previous results were obtained for periodic boundary conditions. We also imposed reflecting and absorbing boundary conditions and found only slight differences. The reflecting and periodic boundary conditions are mathematically identical as long as the dispersal kernel, the resource distribution and the initial adult distribution are symmetrical. When the resource distribution is random or the initial adult distribution is not placed in the centre, the results for these boundary conditions are quantitatively different, but qualitatively similar with respect to persistence after 20 generations. With the absorbing boundary condition, we found only small quantitative differences with the other two boundary conditions for local initial adult distributions ((II) and (III)). For a homogeneous initial adult distribution (I) the absorbing boundary condition gives a qualitatively different result: in almost all cases the reflecting and periodic boundary conditions resulted in extinction whereas the absorbing boundary condition resulted in persistence unless the inter-patch distance (spatially structured resource (2)) is too large or the resource density (spatio-temporally heterogeneous resource (3)) is too small to allow (re)colonisation.

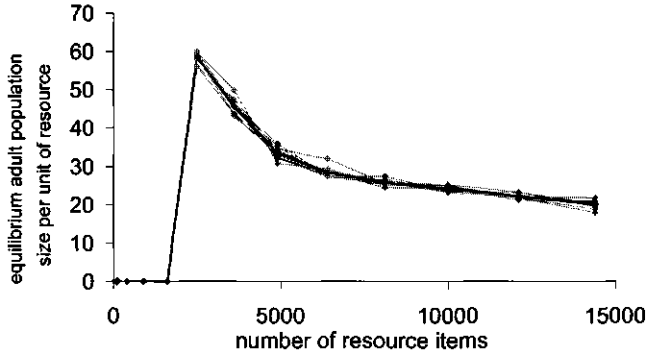

Figure 6: The equilibrium level of the adult population size per unit of resource (i.e. its value at generation 20, see figure 2) versus the number of cells containing resource items for several values of P_0 (grey lines; each line is an average over 10 trials) the average of which is the black line. Dispersal is random, the initial adult distribution is the 256-cell source (III) and the resource distribution is spatio-temporally heterogeneous (3).

Hence in (I), even the small amount of heterogeneity in the adult distribution introduced by the absorbing boundary condition can add sufficient heterogeneity to enable persistence.

Double exponential and ring random dispersal

Because for random dispersal reflecting and absorbing boundary conditions yielded mostly the same results as periodic boundaries, we restricted the simulations for the alternative dispersal kernels to periodic boundaries only. We will present the differences of these alternative kernels with random dispersal.

Double exponential dispersal

The results for double exponential dispersal are similar to those for random dispersal, the main difference being that double exponential dispersal enables establishment and persistence at more values of P_0. The simulated spatial distributions (as in figure 3) suggest that the higher peak, the corresponding faster decline at small distances as well as the fatter tail of the double exponential kernel relative to the random kernel are responsible. The higher peak facilitates establishment at low densities, the faster decline helps persistence, and the fatter tail makes (re)colonisation easier.

Ring random dispersal

A homogeneous initial adult distribution (I) gives a similar result as with random dispersal. One exception to this rule is the spatially structured environment (2) with inter-patch distances larger than 16 m, where populations now can establish and persist at low P_0-values. This seems surprising at first but there are two explanatory mechanisms: dilution (keeping the population density more often between the lower and upper bounds) and (re)colonisation (because the mean displacement is increased due to the additional displacement ρ).

For a local 4-cell source initial adult distribution (II) all simulated situations are doomed to extinction. The cause of this phenomenon is that the fruit flies spread over larger distances than with random dispersal thereby reducing their numbers below the lower bound (too much dilution).

For the 256-cell initial adult distribution (III) ring random dispersal with $\rho = 5$ m results in persistence in a homogeneous environment (1) for every P_0-value; at $\rho = 10$ m persistence occurs only for some P_0. In the spatially structured environment (2) we observed the relationship in figure 7 for $\rho =$ 5 m. For low P_0 very large inter-patch distances seem to be bridged, but closer observation of the simulations reveals that individuals cannot colonise other patches: persistence is entirely due to dilution. At higher P_0-values, however, the effect of dilution cannot ensure persistence adequately. For $\rho = 10$ m we observed a relationship similar to figure 4 with the same threshold inter-patch distance. In this case dilution is either too large ($P_0 \leq 65$) or too small ($P_0 \geq 66$) to enable persistence of a local population: the population will cross the lower or upper bound respectively. The spatio-temporally heterogeneous resource distribution (3) allowed for persistence between 2500 and 14400 occupied cells with some exceptions at $\rho = 5$ m and more exceptions at $\rho = 10$ m, whereas with random dispersal no such exceptions occurred. The probable cause is again dilution.

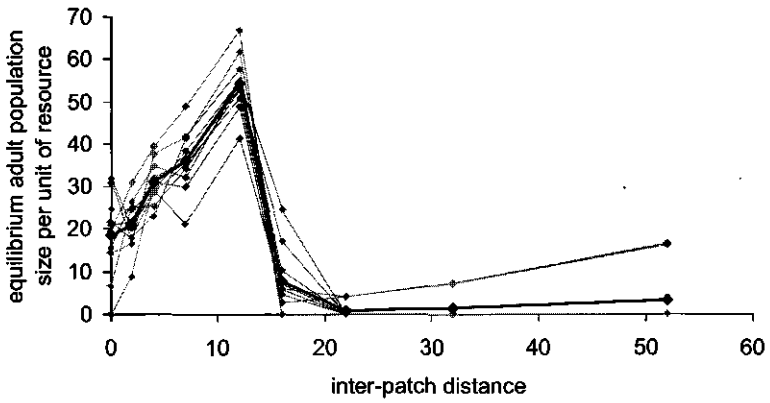

Figure 7: The equilibrium level of the adult population size per unit of resource (i.e. its value at generation 20, see figure 2) versus inter-patch distance for several values of P_0 (grey lines) the average of which is the black line. The initial adult distribution is the 256-cell source (III), the resource distribution is spatially structured (2) with patches of 8 m by 8 m, and the dispersal kernel is ring random with $\rho = 5$ m. Only the two lowest P_0-values (12.5, 25) persist at large inter-patch distances. The lines for these P_0-values increase with inter-patch distance because at larger inter-patch distances the total number of resources decreases while there is only one occupied patch at these distances.

Discussion and conclusion

We found that a variety of simulation conditions determines whether the population can establish and persist. Sufficiently expanded local initial adult populations (e.g. 256-cells) have a high probability of establishment and persistence, whereas both small local and homogeneous initial adult distributions are prone to extinction, because either establishment or persistence is hampered. Establishment and persistence for small local initial adult distributions is greatly influenced by the shape of the dispersal kernel. The only boundary condition we observed to alter population dynamics is the absorbing boundary condition, since a homogeneous initial adult distribution is sufficiently thinned in some regions.

The model presented here was loosely based on the biology of *Drosophila* fruit flies, but possibly applies equally to many insects on ephemeral resources suffering from an Allee effect and scramble competition. Evidently, one can always add more realism (e.g. temporal variation in amount of resource, overlapping generations, stochasticity), but this may be at the expense of generality or give rise to far more complicated models.

In our model, for dispersal to ensure persistence of populations subject to an Allee effect and scramble competition, some form of heterogeneity is needed, either in the resource distribution, the initial adult distribution, the boundary conditions or combinations of these. The interplay between patch size, inter-patch distance and the shape of the dispersal kernel further determines the fate of the population. This conclusion may not be utterly surprising, but is not immediately obvious either given the nonlinearity of the system. Furthermore, closer observation of our results reveals some interesting phenomena.

Although local dynamics were constructed to be massively unstable and non-persistent, the averaged dynamics of the total population were reasonably smooth. The upper and lower local population bounds, coupled with dispersal, created an effectively stable mean population size for the system as a whole, that is, the total numbers of individuals over all resources converged to a steady level. Therefore, this work suggests that stable mean population sizes for spatial populations can be emergent properties that appear at sufficiently large scales, as opposed to inherent properties occurring at all scales.

Spatial models including dispersal are ample in biology (see e.g. Tilman and Kareiva 1997). Often space is introduced explicitly through dispersal to investigate whether it can stabilise unstable local dynamics. A classical example is the Nicholson-Bailey host-parasitoid interaction (Nicholson and Bailey 1935) in which both hosts and parasitoids oscillate wildly until they go extinct. Many mechanisms have been suggested to stabilise these dynamics (e.g. Hassell et al. 1991, May et al. 1981), of which dispersal is a natural one (Hassell et al. 1991). The same holds for many other multi-species interactions (e.g. Sabelis et al. 1991). Yet, for single-species interactions, Nee et al. (1997) notes that dispersal has often no role in stabilising dynamics in homogeneous environments, for example in the intra-species competition model of Hassell et al. (1976). Our results seem to be in accordance with this because, as stated above, some heterogeneity is needed for space to play a stabilising role. However, most single-species spatial models deal only with competition while our model also incorporates the Allee effect, which can nullify the effect of dispersal by preventing colonisation in areas with densities below the lower bound. Our results demonstrate that even with an Allee effect, dispersal may act as a stabilising

factor. In fact, the Allee effect may also help to stabilise by preventing rapid homogenisation of local populations in small patches. In this way, the Allee effect also introduces heterogeneity. Gyllenberg et al. (1999) made a similar suggestion when considering non-local competition in a two-patch metapopulation model.

Dispersal may act as a stabilising factor, yet the *shape* of the dispersal kernel determines the degree of stabilisation to a large extent. The double exponential kernel with its higher but thinner peak and fatter tail seems to be a better dispersal option than random dispersal by ensuring persistence both at a local and at a regional scale (a result not found by Doebeli and Ruxton (1998) in a metapopulation context). This may be an evolutionary explanation of the often observed (e.g., Kot et al. 1996, Neubert et al. 1995) leptokurtic redistribution kernels (kernels that are more pointed than the normal distribution kernel) of which the double exponential kernel is an example. In retrospect the explanation seems to be that the higher peak facilitates establishment at low densities, the faster decline helps persistence, and the fatter tail makes (re)colonisation easier. We expect that these trends will be accentuated by more realistic dispersal kernels, including kernels describing chemotaxis (the active attraction to pheromones or substrate odors). We defer discussion of chemotaxis to later work in which we will apply the method used by Powell et al. (1998).

Not only with the dispersal kernels, but also with the spatial structure of resources and boundary conditions we tried to model a broad spectrum of biologically relevant environments. The different resource distributions create systems at varying spatial scales. In our case study these correspond to a large orchard filled with varying amounts of resources ((1) and (3)) or several small orchards or fruit trees separated by areas without resource (2). The different boundary conditions create systems ranging from open to closed ones (see Outline of the modelling procedure). Each set of boundary conditions may apply depending on the spatial scale: if the simulated area is embedded in a larger structure, immigration approximately equals emigration, so periodic boundaries seem most appropriate; if the simulated area is isolated, reflecting or absorbing boundary conditions are more realistic.

One *caveat* of our analysis might be geometry of the computational implementation: we defined circular (axisymmetric) dispersal kernels on a square grid. This could give rise to artefacts in the corners of the grid: dispersing fruit flies reach the boundaries earlier than the corners resulting in interference patterns of dispersal waves which create heterogeneity. In nature, however, the resulting wave front of dispersing individuals does not match the shape of the habitat either. Therefore, our set-up is likely to capture the essence of dispersal in natural systems.

Another *caveat* is the assumption of all-or-nothing local dynamics. Although we admit that in the field such sharp bounds do not exist, the precise form of the survival probability as a function of local population size is not known in general, and any choice of such a function may be just as arbitrary. Our main objective was to evaluate the stabilising effect of dispersal in conjunction with the Allee effect on a system that is inherently unstable due to scramble competition, a positive growth rate and limited resources. With all-or-nothing dynamics we ensured this in a very simple way. Moreover, all-or-nothing dynamics may not be as unrealistic as it seems. Granted, if there are no strict upper and lower bounds, a local population does not go extinct deterministically. Nevertheless, we argue that stochastic processes, other Allee effects (e.g. in mate finding) and reduced fecundity (due to crowding) still lead to local

extinction. As for our case study on *Drosophila*, laboratory studies (Bakker 1961) show the lethal upper bound. In field studies usually there are at least some survivors, because differences between individuals in for example feeding rates and time of hatching (e.g. due to differences in time of oviposition) favour those who develop fastest. Yet, early hatching may be advantageous as well as disadvantageous to fast development: early hatchers outcompete late hatchers, but only if they are in sufficient numbers not to suffer from the Allee effect which probably act stronger on early hatchers. Thus, differences in time of hatching must not be overestimated. Perhaps their importance only lies in raising the upper bound, because more eggs can be laid upon a resource item. This will hardly affect our qualitative results since this is only a matter of scaling. Moreover, because we used an all-or-nothing rendition of survival within ephemeral resources, we were able to focus clearly on the role played by dispersal in stabilizing an otherwise unstable population.

Acknowledgements

We are grateful to Hans Heesterbeek, Johan Grasman, Marcel Dicke, and Louise Vet for commenting on an earlier version of the manuscript, and to Martin Boer, Marco Bink, Margriet Hendriks and Hans Heesterbeek for allowing us to use their computers for the simulations. We would also like to thank the National Science Foundation (INT-9813421) and the C.T. de Wit Graduate School for Production Ecology of Wageningen University for supporting J.P. during a visit to the Netherlands.

CHAPTER 9

GENERAL DISCUSSION

Towards an ecological cost-benefit analysis of aggregation pheromone use in *Drosophila melanogaster*

Introduction

Aggregated distributions of animals, where individuals actively seek the presence of conspecifics, can only evolve when the benefits to individual group members outweigh all associated costs. Furthermore, this positive balance must already apply at low densities, because evolution proceeds gradually, and cannot skip any major fitness pits that might occur at intermediate densities. Such a situation could arise when survival or reproduction is hampered at low population densities, i.e., with an Allee effect (for a recent definition, see Stephens et al. 1999).

The aggregative behaviour, that arises from individual responses to the presence of others, implies that the individuals release information on their whereabouts, either inevitably by their actions or deliberately, and that others pick up on this and adjust their behaviour in response. When both the sender and the responder benefit from the information conveyance, it is called communication (Bradbury and Vehrencamp 1998). However, the cues that are released by an individual can be exploited by everyone in the food web (Haynes and Birch 1985, Vet and Dicke 1992, Stowe et al. 1995, Dicke and Vet 1999, Dicke and van Loon 2000). In essence, the food web is accompanied by an information web, that may profoundly alter the ecological interactions.

This chapter is a synthesis of all previous chapters on the ecological costs and benefits that are associated with the use of aggregation pheromone in *Drosophila melanogaster*. The central issue of my thesis constitutes the following questions: Why do drosophilids use aggregation pheromone? Which benefits promote this kind of behaviour, and at what costs? What is the optimal behavioural strategy for senders and responders under various ecological conditions?

Males and their charms

Aggregation pheromone in *Drosophila* is produced by males, who thus bear the physiological costs of production (Butterworth 1969, Brieger and Butterworth 1970, Bartelt et al. 1985b). Indirect benefits of the pheromone for males may arise from enhanced offspring survival (see below). The direct benefits of the pheromone for males are twofold.

The first direct benefit for males is the enticement of females (Bartelt et al. 1985b, chapter 3 and 4). Since both virgin females and males need to find a mate to reproduce, enticement serves them both, and can be regarded as co-operation. In accordance with this sexual role for the pheromone, copulations

are frequently observed in *Drosophila* aggregations (Spieth 1974, chapter 3). Conversely, other males are also attracted by the pheromone, and in a field study, increasing aggregation size was correlated with a reduced number of copulations, suggesting competition for mates and increased interference (chapter 3). Clearly, that would not benefit the male sender, but might be unavoidable if the chances of acquiring a mate are smaller when males would 'not call' than when they 'have to share'.

The second direct benefit to males arises from the transfer of the pheromone to females during copulation, together with a load of other accessory gland products. Males engage in chemical mate guarding, which can be considered as a vile act in the battle between the sexes. The male endows his mate with aggressive hormones and toxins, that together must prevent her from re-mating (Wolfner 1997, see also Smid 1997, for a convincing evolutionary argument). The toxins render mating into a costly process, that shortens the females' lifespan (Chapman et al. 1995), whereas the hormones dictate the females to refrain from re-mating. Females that remain faithful live longer, and can achieve a higher fitness than females that are promiscuous, which is a selection pressure on the females to 'obey' the aggressive hormones (Smid 1997). Mate-harming traits can evolve, because the females use the sperm of the last male to fertilize further offspring (Rice 2000). The male anticipates the unfaithfulness of his mate, and abuses the female to fight his male rivals: When he shortens the lifespan of the female, he limits her late reproductive output. Thus, a reduction in the female's life time harms the second male more than the first male, and results in a smaller relative contribution of the second male to the next generation. This comprises a fitness gain to the first male, and the trait can invade in the population. The exact role for the aggregation pheromone as part of the complete ejaculate is not fully unravelled, but may at least function in deterring other males (Zawistowski and Richmond 1986, Costa 1989, Ferveur et al. 1989). When females are coerced into chastity, they may use the pheromone as a reliable signal to avert harassment by other males. New courting males may save energy and time, when the chances are slight that a female will allow a re-mating. Both the mated female and her (first) mate benefit, because she can devote more time to feeding (i.e., egg production, extending the reproductive period) and oviposition.

Women should stick together

Although the females seem to be nastily tricked into fidelity, they have turned the possession of aggregation pheromone to use. Apart from the proposed benefit in averting obtrusive males, who indeed disturb them frequently and may cause a reduced oviposition rate (chapter 3), females use the aggregation pheromone to effectuate aggregated oviposition (chapters 3 and 4). This behaviour was found to enhance the survival and size of their offspring (chapter 5), and can be considered as co-operation, induced by an Allee effect. The adults reduced fungal growth on the resource and enhanced its quality, presumably by inoculation with yeasts. The larval offspring of *Drosophila* feed on the yeasts and bacteria that develop on a resource, whereas fungal growth could antagonise yeast and larval development (chapter 5). Presence of the larvae also diminishes fungal growth although, in our laboratory studies, this yielded no significant benefits to the measured fitness components. Possibly, the larval 'weeding' activity could provide additional benefits when dealing with more noxious fungal species under more natural circumstances (see also Sang 1956, Atkinson 1979, Ashburner 1989, Hodge et al. 1999). Altogether, increased adult densities

enhanced larval survival and growth, but increased larval densities resulted in competition for food (i.e., reduced survival, diminishing size of survivors, increased developmental time). The latter is a cost to aggregated oviposition. Whether the aggregated oviposition by females enhances the resource quality sufficiently to outweigh the negative effects of increased larval competition will in general depend on the characteristics of the resource and the residing micro-biota. Aggregation might reduce mortality only under adverse conditions, whilst the advantage is lost for more benign conditions (Lockwood and Story 1986).

The evolution of signals requires a considerable minimal signal accuracy, because prior to the adoption of communication, animals mostly use alternative strategies (i.e., experience, other cues) that doubtlessly do better than random choices (Bradbury and Vehrencamp 2000). When relying on information provided by others, the fitness gain due to the increase in good choices should compensate for the chance of bad choices because of insufficient accuracy or illicit use. In *Drosophila*, the adults are attracted to volatile fermentation products that emanate from yeasty substrates (Fuyama 1976). This yields a reliable indication of substrate quality. Possibly, the pheromone is particularly desirable to exploit substrates of lesser quality, when communal breeding might enhance substrate quality sufficiently to allow for larval food exploitation of otherwise unsuitable resources. The aggregation pheromone is an accurate indicator of the presence of other gravid females, because females can only obtain it by mating (guaranteeing their mating status) and recently mated females have both a high emission rate of pheromone and a high oviposition rate (Bartelt et al. 1985b, Partridge et al. 1986).

Spies and conspiracies

The use of (chemical) signals in ecological interactions enables espionage. Everyone in the food web can exploit the information, and use it for expediency. This topic has received broad attention for plant-insect-natural enemy interactions and for insects using sex pheromone, but is less integrated in studies on aggregation pheromone (Dicke and Sabelis 1992, Tumlinson et al. 1992, Vet and Dicke 1992, Stowe et al. 1995, Dicke and Vet 1999, Haynes and Yeargan 1999, Dicke and van Loon 2000, chapter 2).

Larval parasitoids home in on the aggregation pheromone of *Drosophila* (Wiskerke et al. 1993a, Hedlund et al. 1996a, chapter 6). In several field experiments, drosophilid larvae in substrates with aggregation pheromone experienced a higher risk of parasitism than in substrates without pheromone (chapter 6). Although aggregation is frequently assumed to be associated with a dilution of risk for increasing densities (*sensu* Hamilton 1971), this appeared not to be the case for the *Drosophila* - *Leptopilina* system, according to our model study and field experiments (chapter 7). The parasitoids adjusted their time-allocation on the substrate in response to host cues and number of ovipositions, and exploited the increased larval densities. This precluded the occurrence of a beneficial effect of clustering with respect to parasitism, and caused a significant increase in the risk for increasing host density (chapter 3 and 7). The aggregation pheromone augmented the risk across all host densities, and thus evokes a cost to its use.

Competitor species, such as *D. simulans*, *D. subobscura*, *D. hydei* and *D. immigrans*, also responded to the pheromone (Bartelt et al. 1986, 1988, Jaenike et al. 1992, chapter 3). They chose significantly more often for substrates treated with the aggregation pheromone of *D. melanogaster* than for control substrates

without pheromone. The main benefit for females, namely enhancing substrate quality for the offspring, is not necessarily species-specific, and could therefore as well be adopted with heterospecifics. Moreover, the interaction between *D. melanogaster* and the fungi even facilitated the resource exploitation for *D. hydei* (Hodge et al. 1999), which would promote species association for the latter. As for the increased larval densities, costs through food competition among larvae could again apply, and these costs may be asymmetrical among the species.

An evolutionary argument

From the male's perspective, for the production of aggregation pheromone to persist, the benefits in acquiring mates, enforcing chastity in their mates and enhanced offspring's survival and growth should outweigh the physiological costs of production and the costs of competition for mates. From the mated female's perspective, for their use of the pheromone to persist, the benefits in reduced harassment and enhanced exploitation of resources by their offspring (i.e., growth and survival) should exceed the reduced oviposition rate through interference in large aggregations, increased larval food competition and increased larval risk of parasitism.

The cost-benefit matrix that can be formulated for these interactions is complicated, because the pay-offs and penalties are mostly density dependent. Furthermore, stochasticity may provide an essential element for the strategy that proves best. For example, different fungi can colonise the larval resource, the chances of finding an alternative breeding substrate vary and so does the resident parasitoid population, and different competitor species can join the aggregation.

Figure 1 depicts a speculative pay-off matrix for mated female *D. melanogaster*. The matrix is loosely based on our findings, and should be considered as a hypothesis. Pay-offs are expressed in the number of offspring that an individual gains or loses when applying the strategy that is denoted on the left of the matrix. Whenever this value is positive, it pays to adopt the strategy. The highest gain is achieved at the peak value, but the strategy should only be abandoned for negative values (i.e., when losses are suffered). The benefits for responders and senders are not equal. For each, a number of habitat characteristics are included in the matrix that are assumed to be of primary importance. It is assumed that different habitat characteristics select for opposing strategies.

Responders (fig. 1a-d) are assumed to be currently without a resource, and the chance to produce *any* viable offspring results in a positive pay-off. The benefits for responding to aggregation pheromone depend on substrate quality, the density of females that are already present on the substrate ('resident density'), and the number of alternative substrates that are available ('substrate density'). The resident's density should be large enough to ensure an Allee effect (i.e., enhanced larval survival and development) when joining, although not too large to prevent severe larval competition. Conversely, the substrate density should be large enough to allow for avoiding an aggregation when the conditions are not ideal. Hence, a female should always join a good quality substrate, unless the resident density is too high to produce good quality offspring (fig. 1a) and substrate density is sufficiently high to take a chance and keep on searching (fig. 1b). For poor quality substrates, a female should only respond when the resident population is sufficiently large (fig. 1c) and the chance of finding another (better) substrate is low (fig. 1d).

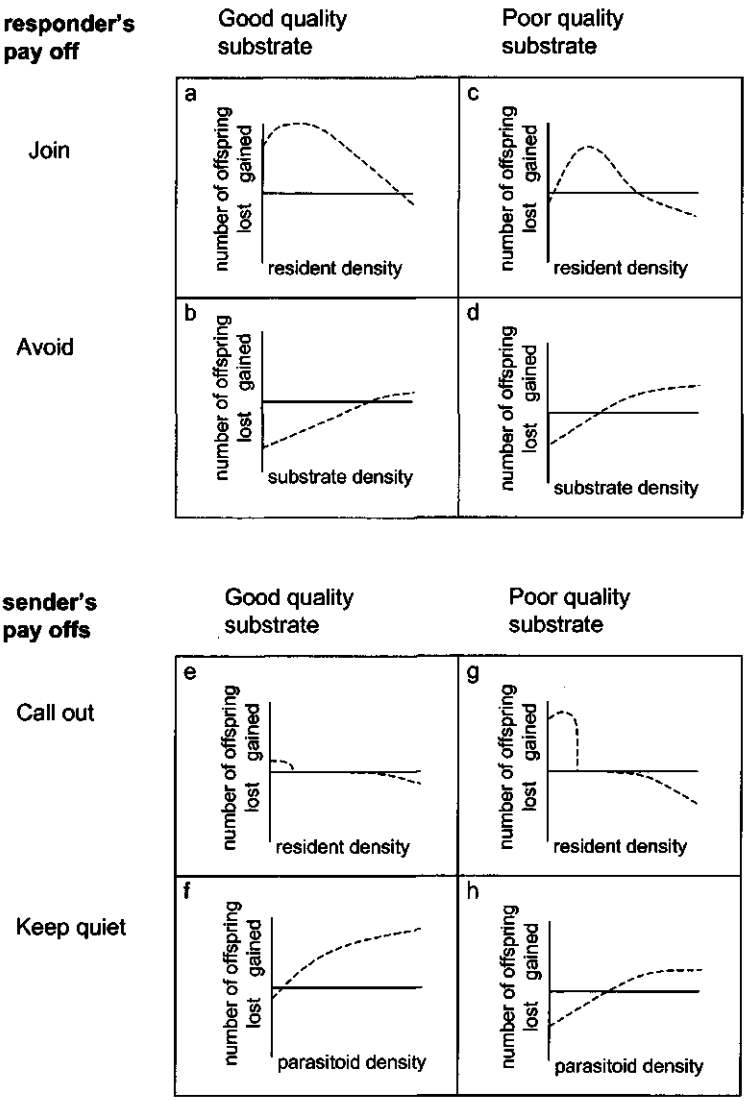

Figure 1: Hypothetical pay-off matrix for mated female fruit flies that can send or respond to aggregation pheromone. The currency is the number of offspring gained or lost when applying the strategy that is denoted on the left of the matrix; whenever this value is positive, it pays to adopt the strategy. The reference points against which these gains and losses are measured differ for responders and senders. Responders are assumed to be currently without a substrate (i.e., the chance to produce *any* viable offspring results in a positive pay-off), whereas senders are assumed to be on a substrate (i.e., the chance to produce *more* viable offspring results in a positive pay-off). It is assumed that different habitat characteristics select for opposing strategies, as indicated by the different titles on the x-axes. The characteristics that are included are the quality of the substrate, density of residents on the substrate, density of alternative substrates and of parasitoids in the habitat. The pay-off patterns are described in the main text.

Senders (fig. 1e–h) are assumed to be on a substrate, and the chance to produce *more* viable offspring results in a positive pay-off. The benefits for emission of aggregation pheromone also depend on substrate quality and the number of females that are already present on the substrate. Since a sender has already accepted the substrate, substrate density is no longer of primary importance, but instead, the sender might mind the density of parasitoids that exploit the cues and threaten her offspring. A female should call for others when an increase in the numbers of residents can enhance larval survival and development, but a female should keep quiet when the risk of attracting natural enemies is large. When aggregation is beneficial even in good quality substrates, she might call out for others at low resident population density (fig. 1e), but only when the parasitoid population is small (fig. 1f). After reaching the optimal aggregation size, calling out is at first neutral (i.e., no increase in survival probabilities of the offspring, but no severe larval competition either) and can become detrimental at higher densities (figs. 1e and g). In poor quality substrates with low resident population size, the female must call out for others to enable larval development on the resource (fig. 1g), even when the parasitoid density is considerable (fig. 1h).

The qualitative predictions might be tested with simple laboratory assays. Behavioural plasticity can be explored to test for functional explanations (Prokopy and Roitberg 2001). The behavioural response of *D. melanogaster* was indeed altered in relation to substrate quality: the responsiveness to applied pheromone was significantly more pronounced on poor quality substrates (chapter 4). This indicates that females have the propensity to join others on poor quality substrates, but are less particular about the presence of others when substrate quality is high. This is in agreement with the qualitative predictions. To derive more quantitative predictions will require the development of a game-theoretical model, that incorporates all factors that may influence the cost-benefit analysis.

The true complexity of reality

The use of aggregation pheromone potentially evokes costs and benefits throughout all food and information web interactions. Ideally, all interactions should be scrutinized to result in a conclusive cost-benefit analysis (Dicke and Sabelis 1992). In reality, the complexity of ecological webs is beyond what can feasibly be investigated. The food web interactions that have been presented here reflect a selection of the main interactions, as inferred from a field experiment (chapter 3). Nonetheless, some omissions deserve mentioning, because they may contribute significantly to the eventual cost-benefit balance. Firstly, the investigations were mainly restricted to one parasitoid species (*Leptopilina heterotoma*), whereas other parasitoids are also involved (Janssen et al. 1988) and these show different responses to the aggregation pheromone of *Drosophila* (Hedlund et al. 1996). Furthermore, *D. melanogaster* larvae have the ability to defend themselves against their parasitoids through encapsulation of the parasitoid's egg, and this ability correlates with a reduced larval competitive ability (Kraaijeveld and Godfray 1997). Since both larval competition and risk of parasitism are density dependent and influenced by the use of aggregation pheromone, the interference between the two might influence the costs and benefits. Finally, the spatio-temporal variability in the environment and the asymmetric competitive interactions with other *Drosophila* species could also result in variation in the costs and benefits. Although we fully appreciate the

true complexity of integrating an information web on food web interactions, we do believe that our selection reflects a prominent part of the cost-benefit analysis.

Future perspectives

For most of the many insect species that possess aggregation pheromone, we lack insight in how these pheromones have evolved and under which conditions their use is the optimal strategy (chapter 2). Combining causal mechanisms, evolutionary arguments and calculations, instigated by behavioural observations, can provide significant progress in this field. By combining state-dependent individual based modelling to construct mutant strategies, and evaluating the invasibility criteria of these strategies in population models, we can predict the outcomes in various ecological situations and gain insight in the evolution of aggregation pheromone.

Information webs exist in any ecological system, and yet, their contribution to ecological processes is poorly understood. The information web affects the dispersal and spread of organisms, the distribution of their natural enemies and competitor species, and consequently, population dynamics of all food web species. Describing odour plume dynamics and chemo-tactic responses in a spatio-temporal context, can provide a valuable tool to investigate the functioning of information webs within a food web context. This is of relevance to a variety of research fields, and might bridge the gap between individual behaviour and especially population dynamics and community ecology.

My main conclusion from this thesis is that aggregation pheromones play an intricate role within a food web context, and a variety of costs and benefits arise through their direct and indirect influences on ecological interactions. The costs and benefits for the use of aggregation pheromone are different for male and female fruit flies and depend largely on the characteristics of the environment. Both aggregative distributions and information webs can fundamentally alter population dynamics in a food web context. Therefore, it is essential to achieve a better insight on the causal mechanisms of aggregative behaviour, the function of this behaviour for the individual and the ecological implications for food web interactions. Such a rigorous integration will not only significantly improve our understanding of the dynamics within ecological systems, but also stimulate the recognition that population dynamics rely heavily on spatio-temporal variability and the behaviours of individuals.

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NEDERLANDSE INLEIDING EN SAMENVATTING

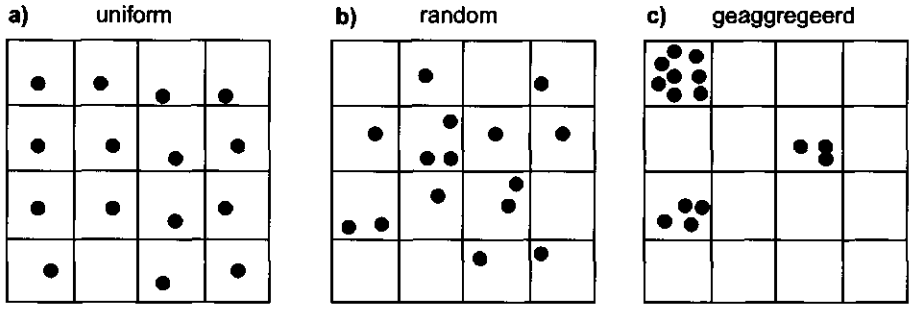
Deze inleiding en samenvatting zijn geschreven voor niet-vakgenoten.
Het is een Nederlandse vertaling van het eerste hoofdstuk,
waarbij vaktaal zoveel mogelijk vervangen is door algemeen taalgebruik.

Evolutionaire ecologie

Het vakgebied van de evolutionaire ecologie omschrijft hoe levende wezenszich fysiek en gedragsmatig aanpassen aan hun leefomgeving. Zo wordt de ruimtelijke verdeling van individuen van een soort over een gebied grotendeels bepaald door de interactie met andere organismen (bijvoorbeeld voedselplanten, soortgenoten, concurrerende soorten en natuurlijke vijanden) en door de kenmerken van het gebied. Deze twee samen beïnvloeden de overlevingskansen en het voortplantingsvermogen van een individu op iedere locatie. Door natuurlijke selectie zijn individuen optimaal toegerust om in te spelen op de omgevingscondities en interacties die ze tegen kunnen komen in hun leefgebied.

Selectie vloeit voort uit de wisselwerking tussen individuen en hun omgeving. Aangezien de omgeving van individuen deels bestaat uit andere individuen, kunnen de interacties met anderen van invloed zijn op het uiterlijk, inwendige processen, en het gedrag van het individu. Bovendien lijkt ieder leefgebied op een geschakeerde lappendeken, die grote ruimtelijke variatie en tijdsgebonden veranderlijkheid vertoont. Samen heeft dat geleid tot een ongelofelijke diversiteit aan strategieën in ecologische interacties. Let wel, al die strategieën zijn gebaseerd op eigenbelang: een bepaalde strategie zal door natuurlijke selectie ontstaan als deze een persoonlijk voordeel oplevert, zelfs als alternatieve strategieën gunstiger zouden zijn voor het algemeen belang.

Dieren kunnen gedragsmatig reageren op de variatie tussen locaties, en kiezen voor de meest gunstige plek die tot hun beschikking staat. Het profijt van een locatie wordt bepaald door, bijvoorbeeld, de hoeveelheid en kwaliteit van het aanwezige voedsel, het risico om er belaagd te worden door natuurlijke vijanden, de aantallen soortgenoten of concurrerende soorten, en de beschikbaarheid van schuilplaatsen om levensbedreigende situaties te ontvluchten. Om in te schatten hoe rendabel een bepaalde locatie is kunnen dieren afgaan op bepaalde aanwijzingen en hun gedrag vervolgens daaraan aanpassen. Desalniettemin, compromissen zijn onvermijdelijk, de aanwijzingen zijn niet onfeilbaar noch volledig en de winstgevendheid van een locatie is niet een statische grootheid maar verandert met de tijd, ook onder invloed van de aantallen en kenmerken van andere organismen. Bovendien, wat de beste strategie is in de ene omgeving kan een slechte keus zijn in een andere. De strategie die gehanteerd wordt door een individu leidt tot zowel kosten als baten, en die kosten en baten variëren in verschillende leefomgevingen.



Figuur 1: Schematische weergave van ruimtelijke verdelingen van organismen. De grote vierkanten stellen het hele leefgebied voor, met daarin verschillende locaties (de vakjes) die identiek verondersteld worden in alle kenmerken behalve het aantal individuen (de stippen). **a)** Als individuen elkaar vermijden, ontstaat een ruimtelijke verdeling die uniform of gelijkmatig is. **b)** Als individuen hun locatie kiezen, onafhankelijk van de aanwezigheid van anderen, ontstaat een random of willekeurige ruimtelijke verdeling. **c)** Als individuen elkaar opzoeken en samenscholen, ontstaat een geaggregeerde of geclusterde verdeling.

Als dieren reageren op de aanwezigheid van soortgenoten bij de keuze van een locatie, kan dat resulteren in een spectrum aan ruimtelijke verdelingen (fig. 1a-c). Het onderwerp van mijn proefschrift is de evolutionaire ecologie van een *geaggregeerde* verdeling van dieren over de omgeving (fig.1c) waarbij de dieren actief hun soortgenoten opzoeken; ze scholen dus samen in groepen. Zowel de geaggregeerde verdeling zelf als het actief reageren op de aanwezigheid van soortgenoten beïnvloedt een scala aan ecologische interacties (hier wordt in een later stadium verder op in gegaan). Bovendien is er een aantal nadelen verbonden aan dit samenscholingsgedrag, en toch blijft het gedrag bestaan. Dat roept verschillende vragen op: Waarom aggregeren deze dieren, hoe is dit gedrag ontstaan en wat zijn de ecologische implicaties voor het individu, de populatie en de hele levensgemeenschap?

Kosten en baten

Als we ons afvragen 'waarom' een organisme een bepaalde eigenschap vertoont, proberen we te achterhalen in welke mate de eigenschap bijdraagt aan de fitness van het individu, ongeacht of de eigenschap betrekking heeft op het uiterlijk, de fysiologie of het gedrag van het individu. Fitness is een relatieve maat die beschrijft hoeveel nakomelingen een individu krijgt, ten opzichte van anderen die een alternatieve eigenschap vertonen. Zoals al vermeld, eigenschappen resulteren zowel in kosten als baten voor degene die de eigenschap vertoont. De evolutie en het voortbestaan van een bepaalde eigenschap vereist dat de baten de kosten overtreffen.

De kosten en baten moeten worden uitgedrukt in een meetbare grootheid, die de cruciale fitness-component beschrijft. Voorbeelden van zo'n grootheid zijn de ontwikkelingssnelheid van de nakomelingen, het aantal en de grootte van de nakomelingen, de overlevingskans per tijdseenheid, of het aantal partners gedurende het hele leven. Het specificeren van de grootheid is gebaseerd op een

hypothese. De hypothese wordt getoetst door het formuleren van specifieke voorspellingen met betrekking tot de eigenschap en de daaropvolgende experimenten die bewijs leveren ten gunste of ten nadele van de hypothese. Meestal worden de kosten en baten beïnvloed door een veelheid aan interacties die, idealiter, alle onderzocht zouden moeten worden om een definitief oordeel te kunnen vellen. In werkelijkheid is de complexiteit van dit netwerk van ecologische interacties te groot om volledig onderzocht te kunnen worden. Doorgaans worden alleen de belangrijkste interacties er uitgelicht.

Bij het onderzoeken van kosten en baten wordt vooraf aangenomen dat individuen datgene doen wat het grootste voordeel voor hen oplevert, rekening houdend met de geldende beperkingen (bijvoorbeeld fysieke beperkingen). De aanname over 'het goede gedrag vertonen' berust op de veronderstelling dat natuurlijke selectie individuen heeft geoptimaliseerd aan de leefomstandigheden. Deze veronderstelling wordt bij een kosten-baten analyse niet getoetst, maar wordt vooraf geaccepteerd. Ongetwijfeld gaat de aanname niet altijd op en daarom is enige voorzichtigheid noodzakelijk bij het interpreteren van onderzoeksgegevens over kosten en baten. Desalniettemin is de analyse van kosten en baten bijzonder bruikbaar voor experimenten. Als de juiste voorzichtigheid in acht wordt genomen kan het een waardevol inzicht verschaffen in de processen die ten grondslag liggen aan de eigenschappen van individuen.

Voor de geaggregeerde verdelingen (zie fig. 1c) die centraal staan in mijn proefschrift kunnen verscheidene kosten en baten van toepassing zijn. Naast aggregaties kunnen de signalen die individuen gebruiken om soortgenoten aan te trekken ook de interacties beïnvloeden met niet-soortgenoten. Het uitgangspunt is dat het vormen van groepen de fitness verhoogt van de afzonderlijke deelnemers, de individuen. Mijn vraagstelling is, *waarom* is dat zo? Welke baten stimuleren groepsvorming en tegen welke kosten?

Het ecologische belang van aggregatiegedrag

Aggregatiegedrag heeft belangrijke implicaties voor de ecologie van dieren; dat wil zeggen samenscholingsgedrag beïnvloedt de relaties die een organisme heeft binnen zijn leefomgeving. Dieren in een groep hebben beduidend meer met groepsgenoten van doen dan met andere individuen en het samenscholen beïnvloedt hun gedrag (bijvoorbeeld hoeveel tijd besteed wordt aan eten, vechten of ouderzorg), hun uiterlijk (bijvoorbeeld grootte of kenmerken die dominantie aangeven) en hun fysiologie (bijvoorbeeld hormoonspiegels). Welbeschouwd beïnvloedt aggregatie de overlevingskansen en het voortplantingssucces van individuen. Op het niveau van de populatie beïnvloedt aggregatie de mate van (voedsel)concurrentie tussen soortgenoten en niet-soortgenoten, de verspreiding en de interactie met natuurlijke vijanden. Geaggregeerde verdelingen creëren variatie in de intensiteit van de interactie tussen soorten. Hoewel een soort in de ene locatie volledig overheerst kan worden door een andere soort, kan in een andere locatie die andere soort afwezig zijn of is die andere soort niet meer superieur. Dit vergemakkelijkt het naast elkaar voortbestaan van verschillende soorten, ofwel concurrenten ofwel natuurlijke vijanden en hun prooien en zodoende bevordert het de biodiversiteit. Anderzijds kunnen geaggregeerde verdelingen leiden tot overexploitatie van de voedselbronnen, snelle verspreiding van besmettelijke ziekten en daardoor tot massale sterfte. Om de dynamiek in ecologische systemen te

begrijpen, te voorspellen en eventueel te sturen is het essentieel om een beter inzicht te krijgen in de rol van aggregatiegedrag van individuen.

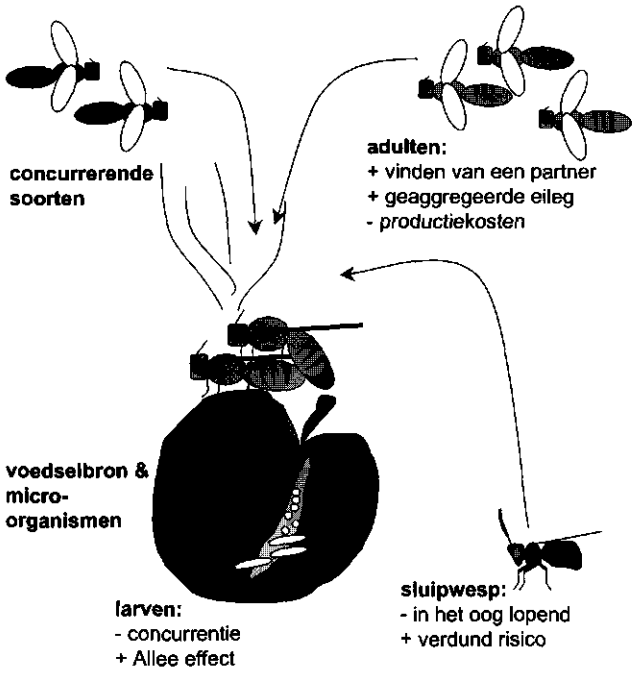
Informatie-overdracht en communicatie

Het door mij onderzochte aggregatiegedrag ontstaat doordat individuen reageren op de aanwezigheid van soortgenoten. Dit houdt in dat individuen informatie vrijgeven over hun verblijfplaats - hetzij ongewild als bijproduct van hun handelingen, hetzij opzettelijk - en dat anderen deze informatie oppikken en hun gedrag er op afstemmen. De signalen die voor informatie-overdracht gebruikt worden kunnen visueel zijn, chemisch (geur en smaak), auditief of tastbaar. De signalen die centraal staan in dit proefschrift zijn aggregatieferomonen. Aggregatieferomonen zijn chemische stoffen, vrijgegeven door een individu, die soortgenoten aantrekken en/of vasthouden op de plek van de verzender. Als zowel de verzender als de ontvanger profiteren van de informatie-overdracht wordt het proces communicatie genoemd. Misbruik van informatie is echter ook denkbaar. De signalen die worden vrijgegeven door een individu kunnen uitgebuit worden door iedereen in het voedselweb. Een voedselweb geeft aan wie door wie gegeten wordt, en wie concurreren om dezelfde voedselbron. Natuurlijke vijanden bijvoorbeeld, kunnen spioneren en vervolgens afgaan op de signalen om hun slachtoffers te vinden en concurrenten kunnen de communicatie 'afluisteren' en hun strategie aanpassen in reactie op die informatie (bijvoorbeeld de voedselbron ontwijken of overmeesteren). In essentie wordt het voedselweb vergezeld door een informatieweb, waardoor ecologische interacties aanzienlijk beïnvloed kunnen worden. Zo'n informatieweb bestaat voor ieder ecologisch systeem, maar hun belang voor ecologische processen is onvoldoende onderkend door ecologen. Het informatieweb beïnvloedt de verspreiding van organismen, de ruimtelijke verdeling van hun natuurlijke vijanden en voedselconcurrenten en derhalve de populatiedynamiek van alle soorten in het voedselweb.

Drosophila als een ecologisch modelorganisme

Voor het bestuderen van de kosten en baten voor het gebruik van aggregatieferomonen met betrekking tot ecologische interacties is een modelsysteem nodig waarin het informatieweb gemanipuleerd kan worden en de voedselwebinteracties bestudeerd kunnen worden in het laboratorium en in het veld. De fruitvlieg *Drosophila* is een ideaal modelorganisme voor zo'n studie. Veel fruitvliegsoorten bezitten aggregatieferomonen waarvan de chemische samenstelling bekend is en die eenvoudig toegediend kunnen worden in experimentele opstellingen. Deze insecten zijn algemeen voorkomend, makkelijk te kweken en zeer geschikt om mee te werken in het laboratorium en het veld en hun basale voedselwebstructuur is bekend.

De fruitvliegsoort die gebruikt is in deze studie, *Drosophila melanogaster*, vormt aggregaties op gistend fruit met behulp van aggregatieferomonen. Deze vruchten zijn voedsel- en broedsubstraten. De vliegen eten, paren en leggen eitjes in de gevormde aggregaties. De larven (maden) en adulten (volwassen vliegen) eten voornamelijk van de gistcellen en bacteriën die groeien op de vrucht. De larven hebben, als gevolg van het gebruik van aggregatieferomonen door de adulten, ook een geaggregeerde verdeling over vruchten. In de vrucht concurreren de larven vaak sterk om voedsel. De chemische samenstelling van de



Figuur 2: De informatieweb- en voedselweb-interacties in het *Drosophila* systeem, en de mogelijke kosten (-) en baten (+) voor *D. melanogaster* die ontstaan door het gebruik van aggregatieferomoon. Adulte mannetjes en niet-maagdelijke vrouwtjes geven het feromoon af als ze op een voedsel- en broedsubstraat (vrucht) zijn. Soortgenoten verkiezen substraten met feromoon boven substraten zonder feromoon (zie ook fig. 1). De adulte fruitvliegen in de aggregaties eten, paren en leggen eitjes. Hierdoor zijn ook de larven geaggregeerd op de (paar) substraten waar adulte fruitvliegen zich hebben opgehouden (zie ook fig. 1). De larven eten van de gisten die groeien op de vrucht. Als de larvale dichtheden hoog zijn, concurreren de larven onderling om voedsel. Als de larvale dichtheden laag zijn kunnen er problemen ontstaan met het exploiteren van de vrucht, met name omdat er veel schimmelgroei gaat optreden, ten koste van de gisten. Grotere aantallen larven zijn beter in staat deze schimmelgroei in te perken (dit is een 'Allee effect'). Niet alleen soortgenoten worden aangetrokken door het gebruik van aggregatieferomonen maar ook concurrerende soorten en sluipwespen. De concurrerende soorten leggen ook eitjes op de vrucht, en hun larven kunnen meewerken aan het ontginnen van de voedselbron (gezamenlijk voordeel of in managersjargon een 'win-win situatie'), maar ze kunnen ook de voedselconcurrentie verhevigen. De sluipwesp spioneert op de communicatie tussen adulte fruitvliegen, en door het feromoon kan ze de larven, haar slachtoffers, makkelijker vinden. Daar staat tegenover dat, in theorie, de aggregatie van larven ook het risico op parasitering kan verdunnen. Als een sluipwesp slechts een beperkt aantal van de aanwezige larven aanvalt, wordt de kans gegrepen te worden kleiner als het aantal larven toeneemt.

aggregatieferomonen van *Drosophila* is behoorlijk soortspecifiek, en andere fruitvliegsoorten die in dezelfde leefomgeving voorkomen kunnen aan de hand van de verschillende feromonen soortgenoten en niet-soortgenoten onderscheiden en deze informatie gebruiken voor het kiezen of ontwijken van voedsel- en broedsubstraten. De voornaamste natuurlijke vijanden van fruitvliegen zijn sluipwespen. De

moeder-sluipwesp legt een eitje in een fruitvlieglarve; dat wil zeggen, ze parasiteert de larve en de laatste wordt dan de gastheer genoemd. De fruitvlieglarve wordt gedood terwijl het sluipwesp-jong haar van binnenuit opeet. Om de fruitvlieglarven te vinden, spioneert de moeder-sluipwesp op de communicatie van de adulte fruitvliegen. Dus, doordat de adulte fruitvliegen aggregatieferomonen verspreiden, worden hun nakomelingen makkelijker gevonden door hun natuurlijke vijanden (ze lopen meer in het oog).

Samenvattend, het gebruik van aggregatieferomonen door de adulte fruitvliegen resulteert in duidelijke kosten: De larven ondervinden meer concurrentie om voedsel en worden makkelijker gevonden door hun natuurlijke vijanden. Dus waarom gebruiken fruitvliegen een aggregatieferomoon? Welke baten zijn voldoende om deze kosten te overtreffen? Heeft het te maken met het vinden van een partner, de overleving en ontwikkeling van de larven, of is het een combinatie van deze factoren (zie fig. 2)?

DOEL EN OPZET VAN HET PROEFSCHRIFT

Het doel van dit proefschrift is het bevorderen van het begrip van de ecologische en evolutionaire aspecten van het gebruik van aggregatieferomoon in insecten. Zoals hiervoor is beschreven kunnen aggregatieferomonen grote implicaties hebben voor een verscheidenheid aan ecologische interacties doordat ze een informatieweb verschaffen en groepsvorming veroorzaken. Door het ontleden van de kosten en baten die voortvloeien uit het gebruik van aggregatieferomoon in *D. melanogaster* worden de consequenties van een informatieweb op voedselwebinteracties uiteen gezet. De belangrijkste interacties die door aggregatieferomonen beïnvloed worden zijn geselecteerd in een veldstudie (hoofdstuk 3), en de onderzoeken naar kosten en baten zijn tot deze interacties beperkt.

Hoofdstuk 2: In een literatuuroverzicht worden het bestaan, de baten en de kosten van het gebruik van aggregatieferomonen bij insecten beschreven. Aggregatieferomonen zijn bekend voor meer dan 250 insectensoorten in 10 verschillende insectenorden (bijvoorbeeld kevers, oorwormen, kakkerlakken, bladluizen, sluipwespen en vliegen). Het gebruik van een aggregatieferomoon is dus wijdverbreid onder insecten. De informatie-overdracht is afhankelijk van verschillende factoren die gedetailleerd onderzocht zijn. De baten van aggregatieferomonen zijn veel minder goed bekend, maar voor de meeste soorten kan een voorzichtige indeling gemaakt worden in één of meer van de volgende categorieën, namelijk 1) verhoogde efficiëntie in het ontginnen en exploiteren van een voedselbron; 2) het vinden van een partner; 3) bescherming tegen natuurlijke vijanden; 4) bescherming tegen ongunstige omgevingsfactoren of 5) geaggregeerde eileg. Een aantal kosten wordt ook besproken in het literatuuroverzicht. Het overzicht laat opvallende overeenkomsten zien tussen aanverwante soorten onderling (dit suggereert voorouderlijke aanleg) en tussen niet-verwante soorten (dit suggereert herhaalde evolutie). De nadruk in de meeste studies over aggregatieferomonen ligt op de factoren die informatie-overdracht beïnvloeden, de chemische samenstelling van het feromoon en de mogelijke toepassingen voor plaagbestrijding. De schaarste aan gecombineerde studies naar de ecologische, functionele en evolutionaire aspecten van het gebruik van een aggregatieferomoon belemmert het begrip van het ecologisch belang van dit communicatiegedrag. Het vervolg van dit proefschrift behandelt juist die aspecten voor aggregatieferomonen in *Drosophila*.

Hoofdstuk 3: In een veldstudie is onderzocht welke gedragingen en ecologische interacties beïnvloed worden door het gebruik van aggregatieferomoon in *D. melanogaster* en in welke mate dit van belang is binnen het volledige netwerk van ecologische interacties. Specifiek is gekeken naar de rol van aggregatieferomoon in het kiezen van voedsel- en broedsubstraten, het eileggedrag, het gedrag op een substraat en de interacties met soortegnoten en met niet-soortgenoten. Het feromoon veroorzaakt zowel directe effecten (de ruimtelijke verdeling van adulten, eieren, concurrerende soorten en natuurlijke vijanden) en indirecte effecten (meer hinder voor de adulten door de grote toevloed, meer voedselconcurrentie voor larven). Beide soorten effecten kunnen kosten en baten opleveren voor de fruitvliegen. Het informatieweb en het voedselweb voor fruitvliegen zijn in deze studie geanalyseerd en de belangrijkste interacties die beïnvloed worden zijn geïdentificeerd (fig. 2). Deze interacties worden uitvoeriger beschreven in het vervolg van het proefschrift.

Hoofdstuk 4: Flexibiliteit in het gedrag stelt een individu in staat om zich aan te passen aan verschillende omstandigheden en de optimale strategie te kiezen in reactie op een veranderde kosten-baten situatie. Om te verkennen welke kosten en baten verbonden zijn aan het gebruik van aggregatieferomoon zijn verschillende aspecten van de flexibiliteit in het gedrag van *D. melanogaster* onderzocht met keuze-experimenten in het laboratorium. Aan de hand van de veldexperimenten (hoofdstuk 3) zijn twee hypotheses geformuleerd met betrekking tot een voordeel van het gebruik van aggregatieferomoon. Eén hypothese heeft betrekking op een voordeel voor de adulte vrouwtjes, waarbij aggregatie kan leiden tot een reductie in ongewenste intimiteiten van mannetjes en daardoor een hogere eilegsnelheid; de andere heeft betrekking op een voordeel voor hun nakomelingen, waarbij groepen larven beter in staat zijn om lastige voedselsubstraten te ontginnen. De keuze van adulten voor substraten met aggregatieferomoon was aanzienlijk lager voor hoogwaardige voedselsubstraten dan wanneer dit getest werd met inferieure voedselsubstraten. Dit is een ondersteuning voor de tweede hypothese, die betrekking heeft op een voordeel voor de larven. Het kan betekenen dat het samenbrengen (aggregeren) van de nakomelingen het exploiteren van lastige voedselsubstraten vergemakkelijkt, terwijl voor hoogwaardige voedselsubstraten aggregatie minder nodig is.

Hoofdstuk 5: De evolutie van het gebruik van een aggregatieferomoon vereist dat een individu profiteert van samenscholing. Zo'n situatie kan ontstaan als reproductie of overleving wordt beperkt bij een lage populatiedichtheid (= bevolkingsdichtheid). In de biologie wordt dit fenomeen 'Allee effect' genoemd. Om de mogelijke positieve effecten van aggregatie, de drijvende kracht achter het Allee effect dus, op de overleving en groei van fruitvlieglarven te ontmaskeren, zijn larven opgekweekt bij verschillende dichtheden en werd hun ontwikkeling gevolgd. Ook is onderzocht of de aanwezigheid van de adulte vliegen een toegevoegde waarde had voor de larven. Dit werd onderzocht door verschillende aantallen adulten vooraf op het kweeksubstraat te huisvesten. Bij hogere larvale dichtheden werd slechts een hogere voedselconcurrentie geconstateerd dus geen positief effect van aggregatie. Daarentegen verbeterden de overleving en groei van de larven bij hogere adulte dichtheden voorafgaand aan het opkweken van de larven. Dit ondersteunt de hypothese dat er aanzienlijke baten verbonden zijn aan geaggregeerde eileg in fruitvliegen, via een positief effect op de larvale ontwikkeling. Dit kan toegeschreven worden aan een

interactie tussen de adulten, micro-organismen (schimmels en gisten) en de larven. De larven van *D. melanogaster* eten van de gisten die op een vrucht groeien, maar schimmels remmen de groei van zowel gisten als larven. Adulte vliegen brengen zelf gisten aan op vruchten en verminderen bovendien de groei van schimmels. Hogere adulte dichtheden op een vrucht verhogen dus de kwaliteit van het substraat voor de ontwikkeling van de larven.

Hoofdstuk 6: De sluipwesp *Leptopilina heterotoma* spioneert op de aggregatieferomonen van fruitvliegen. De moeder-sluipwesp wordt aangetrokken door substraten waar het fruitvliegen aggregatieferomoon op zit, en dit kan haar helpen bij het vinden van haar slachtoffers. In deze studie is het effect van het feromoon op de verschillende onderdelen van het zoekgedrag van de sluipwesp onderzocht in laboratorium- en veldexperimenten. De resultaten laten zien dat de reacties van de sluipwesp op het feromoon zodanig zijn dat ze al vanaf een afstand een kwantitatief onderscheid maakt tussen substraten die voor haar verschillen in kwaliteit. Ze reageert bijvoorbeeld op verschillen tussen het aantal fruitvliegen dat eitjes heeft gelegd op het substraat. Dit kan de sluipwesp aanzienlijk schelen in tijdsverspilling aan substraten van mindere kwaliteit. Nadat ze op een substraat gearriveerd is spelen de aggregatieferomonen nauwelijks nog een rol in het vervolg van het zoekgedrag naar de fruitvlieglarven; de sluipwesp schakelt over op andere aanwijzingen om te bepalen waar en hoelang ze gaat zoeken op het substraat. In verschillende veldexperimenten werd een groter percentage van de larven geparasiteerd als die opgroeiden in broedsubstraten met aggregatieferomoon dan in broedsubstraten zonder aggregatieferomoon. Dit toont aan dat er ecologische kosten verbonden zijn aan het gebruik van aggregatieferomoon door fruitvliegen, door een toename van het risico op parasitering van de nakomelingen.

Hoofdstuk 7: Sluipwespen kunnen aggregatieferomonen uitbuiten voor het vinden van hun slachtoffers, maar die laatsten kunnen zich tegelijkertijd verschuilen in een 'kudde' waardoor de individuele kans om geparasiteerd te worden afneemt. Het verschuilen in een kudde wordt vaak genoemd als een verklaring voor het 'waarom' van samenscholingen in dieren. Het verlaagde risico kan veroorzaakt worden door bijvoorbeeld een verlaagde efficiëntie van de sluipwespen doordat de sluipwespen tijd verspillen aan larven die al geparasiteerd zijn, of doordat er meer larven zijn dan een sluipwesp kan aanvallen. Deze voordelen van samenscholing kunnen echter teniet worden gedaan door flexibiliteit in het gedrag van de sluipwesp in reactie op de dichtheid van de larven. Om het individuele risico voor larven in aggregaties te voorspellen moet een aantal zaken gecombineerd worden: de grotere aantallen sluipwespen die afkomen op de aggregatieferomonen, de eventueel verlaagde efficiëntie van de sluipwesp en de flexibiliteit in het gedrag van de sluipwesp. Om te bepalen of en wanneer aggregatie gunstig is voor individuele larven met betrekking tot het risico op parasitering is voor dit onderzoek een simpel wiskundig model ontwikkeld, gebaseerd op het gedrag van de sluipwesp. De voorspelling van het model is dat aggregatie niet gunstig is voor *Drosophila* in de relatie met *Leptopilina* en dat het gebruik van aggregatieferomoon het risico vergroot op parasitering bij alle larvale dichtheden. De resultaten van een eerder uitgevoerd veldexperiment (beschreven in hoofdstuk 3) waren kwalitatief in overeenstemming met deze voorspelling: het individuele risico op parasitering nam toe met de dichtheid van de larven. Dus, het gebruik van aggregatieferomoon genereert ecologische kosten met betrekking tot het risico op parasitering. De baten voor het

samenscholingsgedrag en het 'waarom' voor aggregeren moeten in andere verklaringen gezocht worden dan het schuilen in een kudde.

Hoofdstuk 8: Het gebruik van aggregatieferomoon is een ruimtelijk proces. Het beïnvloedt de verspreiding van individuen en veroorzaakt variatie in de dichtheden op verschillende locaties. Met een wiskundig simulatiemodel is onderzocht welke gevolgen verschillende verspreidingspatronen, voedselconcurrentie en een Allee effect (zie uitleg bij hoofdstuk 5) hebben op de populatiedynamiek. Het model is in zekere zin gebaseerd op *Drosophila*, maar toch vooral zo simpel mogelijk gehouden. Het model voorspelt dat het vestigen en voortbestaan van een fruitvliegenpopulatie afhankelijk is van de oorspronkelijke verdeling van de adulten, de beschikbaarheid van voedsel- en broedsubstraten, de mogelijkheid om die substraten te bereiken en de mate van ruimtelijke variatie. Het model is een eerste stap op weg naar een uitgebreider model waarin ook de reactie van insecten op de ruimtelijke verdeling van substraten en chemische informatie (bijvoorbeeld die van aggregatieferomonen) wordt opgenomen.

Hoofdstuk 9: Het laatste hoofdstuk is een synthese van alle voorafgaande hoofdstukken over de kosten en baten die verbonden zijn aan het gebruik van een aggregatieferomoon in *D. melanogaster*. Een voorstelling van de evolutionaire ontstaansgeschiedenis en de ecologische implicaties van het gebruik van het aggregatieferomoon worden besproken, en er worden suggesties gedaan voor toekomstig onderzoek. Mijn voornaamste conclusie van dit proefschrift is dat aggregatieferomonen een complexe rol spelen in de context van een voedselweb, en een veelheid van kosten en baten doet ontstaan door de directe en indirecte effecten die ze hebben op ecologische interacties. De kosten en baten verbonden aan het gebruik van aggregatieferomoon verschillen voor mannetjes en vrouwtjes en worden in grote mate bepaald door de omgevingsfactoren. Of fruitvliegen het aggregatieferomoon zouden moeten gebruiken hangt in grote mate af van de aantallen en kwaliteit van voedsel- en broedsubstraten en het risico op het aantrekken van sluipwespen en voedselconcurrenten. Om de kloof te kunnen dichten tussen individueel gedrag en ruimtelijke populatieprocessen is het essentieel om onderzoek naar de oorzaak (het 'waardoor') en het motief (het 'waarom') van gedrag te combineren met onderzoek naar de ecologische gevolgen binnen een voedselweb.

NAWOORD

Voor mij is de periode van mijn promotie-onderzoek een fantastische tijd geweest, en dat komt voor een groot deel ook door de mensen waarmee ik in deze periode heb samengewerkt.

Allereerst wil ik mijn promotoren - Louise Vet, Marcel Dicke en Joop van Lenteren - bedanken voor de wijze waarop ze mij hebben begeleid. Louise en Marcel, door jullie warme betrokkenheid, het mee-leven met alle ups en downs, de levendige discussies over het onderzoek en jullie aanstekelijke enthousiasme, samen met jullie vertrouwen, zelfs op de momenten dat het onderzoek allerminst soepel verliep, waren jullie voor mij de ideale begeleiders. Altijd als het nodig was waren jullie er voor me. Ook in de schrijffase heb ik veel van jullie geleerd als mijn stukken telkens met grote snelheid terug kwamen, grondig van commentaar voorzien. Jullie zijn geweldige mensen. Bedankt! Joop hield op afstand de zaak goed in de gaten, en hem wil ik ook bijzonder bedanken voor het lezen en becommentariëren van de laatste versie van de hoofstukken. Daarnaast was de vanzelfsprekendheid waarmee hij geholpen heeft bij het verkrijgen van de verlenging, voor mij van grote waarde en tekenend voor zijn verbondenheid met de promovendi op Entomologie.

In de tweede plaats wil ik heel Entomologie bedanken. De goede en dynamische sfeer op het lab, de gezelligheid in de pauzes en op de regelmatige feestavonden en activiteiten, de vele mensen die met je mee willen denken over ideeën en praktische oplossingen, maken van Entomologie een buitengewoon stimulerende en plezierige werkplek. Ik dank iedereen voor hun kameraadschap en hun hulp en wil een aantal mensen in het bijzonder bedanken: Van de parasietenlunch-groep bedank ik Matthijs Vos, Joep Burger, Clemens Stolk en Remco van Poecke voor het vele malen mee-denken en discussieren over ideeën, proeven, resultaten en tegenslagen. Ook bij Marieta Braks, Conny Schütte en Astrid Groot heb ik heel wat keren advies gezocht en hun ervaringen waren een echte steun bij het ontwikkelen van de bio-assays. Hans Smid was direct bereid om de foto's te maken voor de omslag van mijn proefschrift. Mijn kamergenoten, achtereenvolgens Olga Krips, Frans van Alebeek en Petra Schneider, zorgde voor de dagelijkse gezelligheid. En niet in de laatste plaats de aio-lunch, waarin we elkaars manuscripten uitvoerig becommentariëren en bespreken. Dit is voor mij de letterlijke verwezenlijking van een 'vriendendienst'. Bedankt allemaal!

Henk Snellen en Leo Koopman hebben niet alleen het merendeel van de kweken voor hun rekening genomen, maar waren ook de personen bij wie je altijd terecht kon voor de oplossing van een praktisch probleem of voor een opbeurend woordje. Ook Herman Dijkman, Frans van Aggelen en André Gidding waren altijd bereid 'even' te helpen. Willie Herder en Gradus Leenders hebben me meerdere malen geholpen met het ontwerpen en vervaardigen van proefopstellingen; Anja van Aggelen heeft meer dan 25 strekkende meter stof tot een grote populatiekooi omgevormd. Bij Ans Klunder, Ineke Kok, Marieke Bosman, Riet Tilanus, Sabine Meijerink, Truus de Vries en Wilma Twigt kon je altijd terecht voor het regelen van alles wat geregeld moest worden. De mensen van Tupea hebben mijn computer weten te redden van een aantal bijna-dood ervaringen. Zonder deze mensen die de zaken draaiend houden stort de hele groep in. Bedankt voor al jullie hulp.

Naast de entomologengroep in Wageningen heb ik ook kunnen steunen op mijn Leidse basis. Zij hebben ook tijdens mijn studie al mijn interesse voor dit deel van de ecologie gestimuleerd. Bovendien heb ik met Jacques van Alphen, Jan Sevenster, Gerard Driessen en Coenraad Krijger regelmatig ideeën kunnen uitwisselen over het project. Bedankt dat ik altijd bij jullie langs kon komen en mocht profiteren van jullie inzichten over aggregatie en ecologie.

Voor advies over de statistische verwerking van mijn gegevens kon ik telkens weer terecht bij Gerrit Gort. Bedankt voor het beantwoorden van mijn vele vragen, voor het zoeken naar methodes die het meest geschikt waren voor mijn data en mijn doelen, en voor de koffie bij mijn bezoeken aan het wiskundegebouw. Rob Nout heeft me waardevolle adviezen gegeven over het gebruik van gisten in mijn experimentele substraten. René Boom en Kees Sol zijn speciaal naar Wageningen gekomen om mijn methodologische problemen met de opstellingen eens nauwkeurig onder de loep te nemen, en hun suggesties zijn doorslaggevend geweest voor de verdere opzet van de bio-assays. Yde Jongema heeft me geholpen bij het determineren van de insecten. De expertise van al deze mensen was onmisbaar voor het goede verloop van mijn project.

De studenten die aan mijn project hebben gewerkt, Erik-Jan van Baalen, Anton van der Sommen en Julien Marchais, hebben ieder een belangrijke bijdrage geleverd aan dit proefschrift. Hoofdstuk 2 was er niet geweest zonder Erik-Jan en zijn talent om van de vele stukjes informatie een levendig betoog te maken. Anton heeft met volharding en enthousiasme een opstelling ontwikkeld voor de fruitvliegen, waar ik veel profijt van heb gehad voor hoofdstuk 4. Julien assisted on the laborious work for the first experiment of chapter 5, and kept me alert with his questioning mind. Ik heb genoten van het samenwerken met jullie.

When I intended to do field experiments with *Leptopilina*, I mailed around to enquire where I might be able to do so. Everyone I mailed advised me to contact Michel Boulétreau, and he himself immediately and kindly invited me to his laboratory in Lyon, France. Roland Allemand organised my stay at the INRA field station in Gotheron, and together they generously provided expertise knowledge and research equipment to set off the experiments smoothly. During my stay in Gotheron, I visited them in Lyon on several occassions, and the warm hospitality and stimulating discussions with all members of their research group provided a stimulus for further collaboration. Thank you for all your advice and suggestions. Mr. Marboutie kindly hosted me on the premisses of his field station, and Vincent Mercier and Freddy Combe provided research facilities and practical solutions to every small problem. The work I was able to do on the INRA fieldstation was essential for my thesis and I'm very gratefully to you all.

During his visit and course in Wageningen, Jim Powell boosted my project with a new perspective: spatial modelling. With his vibrant teaching and broad ecological interest, he set in motion a close collaboration with Rampal Etienne, Lia Hemerik, Petra Schneider and me. The (first) results are presented in chapter 8. Hopefully, this collaboration will continue in the near future, and I hope I may benefit from your expertise and patient explanations for a bit longer. Thank you for all you have taught me and for the lively meetings on bugs and space.

De samenwerking met Rampal en Lia is uitgegroeid tot veel meer dan dat eerste project. Ik heb veel geleerd van al jullie feedback op mijn wiskundige inspanningen en bedank jullie voor alle uren die we samen gewerkt en gepraat hebben en voor jullie vriendschap. Het model in hoofdstuk 7 is een

gezamenlijke onderneming met Rampal geweest, waarvan ik onnoemelijk veel geleerd en genoten heb. Lia heeft zich sterk ingezet voor het verwerven van een vervolgproject en het zou fantastisch zijn als we de komende jaren samen verder kunnen werken.

Behalve deze schare van mensen die via mijn werk een belangrijke rol hebben gespeeld, wil ik ook mijn vrienden en familie bedanken voor alles wat ze de afgelopen jaren voor me betekend hebben. De contacten met jullie waren van groot belang om het juiste perspectief te houden en te genieten van het leven buiten het promotie-onderzoek. In het bijzonder bedank ik mijn vrienden van biologie (Sandra, Irene, Sue, Sander en Bas), het dispuut (Anske, Matthijs, Daniëlle, Dimitri, Frederik, Muriël, Marko, Monique, Pier, Clementine, Ronald en Kamiel) en van nog vroeger (Marina, Annemieke en Nellie), voor alle gezelligheid, morele steun, vriendschap en vrolijkheid, en voor hun begrip als ik 'even' niet sociaal was.

Van mijn familie begin ik bij mijn ouders, Pauline en Hugo Wertheim, die op een heel directe wijze betrokken zijn geweest bij mijn promotie-onderzoek. Ze hebben me gestimuleerd altijd datgene te doen wat het leukste was, vertrouwen gehad als het allemaal wat zorgelijk leek, meegedacht over problemen en ideeën voor mijn onderzoek, uren geluisterd als ik mijn verhaal kwijt moest en me op alle mogelijke manieren geholpen, van het uitlenen van computers en het ruilen van auto's in Frankrijk tot het gedetailleerd redigeren van mijn Nederlandse samenvatting. Lieve pap en mam, bedankt! Ook de rest van de familie, Bauk, Nils, Eus, Lidy, Jur, Mieke, Jan, opa, Riet, Leen, Frank en Lucia hebben met warme belangstelling de vorderingen en tegenslagen van het onderzoek op de voet gevolgd. Bauk waakte zorgvuldig over mijn ontspanning en wist als geen ander mijn zorgen te relativeren. Ik zou willen dat Mieke en opa er nog bij hadden kunnen zijn. Bedankt allemaal voor alles!

Tot slot, Erik, jij hebt als geen ander de afgelopen jaren met me meegeleefd, zowel op de leuke als minder leuke momenten. Je hebt me gesteund, aangemoedigd, geholpen, opgevangen en mijn vorderingen gevierd, mijn zorgen en twijfels aangehoord en me gerustgesteld. De vakanties met ons tweeën geven me energie voor een heel jaar, en zonder jou was het niet gelukt. Ik bedank je voor dit alles, maar vooral voor je liefde.

Bregje Wertheim
Rhenen, 20 juli 2001

CURRICULUM VITAE

Op 6 augustus 1971 werd ik, Bregje Wertheim, geboren in Amsterdam. Na het behalen van mijn VWO diploma aan de RSG Broklede te Breukelen in 1989 heb ik een jaar rondgereisd en gewerkt in Nieuw Zeeland. Daarna ben ik in 1990 biologie gaan studeren aan de Rijks Universiteit Leiden. Als onderdeel van mijn studie heb ik gedragsonderzoek uitgevoerd naar de visuele vermogens van twee cichlidensoorten die leven op verschillende diepten, onder begeleiding van Steven Smit, Gerrit Anker en Cees Barel. Voor een volgend afstudeerproject heb ik vanuit het biologsich veldstation in Wijster paddestoelen verzameld en de levensgemeenschap beschreven van insecten die op de paddestoelen leeft. Door in een model de invloeden van verschillende coëxistentie-mechanismen te variëren, kon worden aangetoond dat spatiële aggregatie van soorten noodzakelijk en toereikend was om de coëxistentie te verklaren in de levensgemeenschap van fungivore insecten. Deze studie is uitgevoerd onder begeleiding van Jan Sevenster, Irene Eijs en Jacques van Alphen. Vervolgens heb ik in een afstudeerproject de fitness van kleurvariëteiten van een zweefvlieg vergeleken in veld- en semiveldproeven, onder begeleiding van Mart Ottenheim en Paul Brakefield. Mijn laatste afstudeerproject heb ik uitgevoerd aan het Imperial College in Silwood park, Engeland, onder begeleiding van Christine Müller and Charles Godfray. Daar heb ik met veldexperimenten dichtheidsafhankelijke processen in de populatiedynamica van bladluizen bestudeerd en de invloeden van mutualistische mieren op de overleving van bladluizen onderzocht. In 1996 ben ik cum laude afgestudeerd in de biologie.

Van oktober 1996 tot mei 2001 was ik aangesteld als onderzoeker in opleiding (OIO) aan de Wageningen Universiteit bij het Laboratorium voor Entomologie, op een project gefinancierd door NWO-ALW. Als onderdeel van het project heb ik in 1999 veldexperimenten uitgevoerd in Frankrijk, in samenwerking met Roland Allemand van de Université Claude Bernard, Lyon, Frankrijk. Het promotieonderzoek is uitgevoerd onder begeleiding van Louise Vet, Marcel Dicke en Joop van Lenteren. De resultaten van het project zijn gepresenteerd in dit proefschrift.

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The research described in this thesis was carried out at the Laboratory of Entomology of the Wageningen University. The project was supported by the Council for Earth and Life Sciences (ALW), which is subsidized by the Netherlands Organisation of Scientific Research (NWO), under project number 805-36-342.

Cover photographs by Hans M. Smid, Laboratory of Entomology, Wageningen University.