EXPERIMENTAL APPROACHES TO UNRAVEL PROXIMATE MECHANISMS OF PARASITOID SEARCHING AND PATCH LEAVING BEHAVIOUR

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Doctoral thesis

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DOCTORAL THESIS
by
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Summary

Resources distributed in spatially distinct manners require predators to develop efficient strategies in order to be able to exploit them to the optimum. Their way of life makes parasitoid insects excellent model organisms for the study of such strategies. Adult female parasitoids search for suitable hosts for oviposition, which are, different insect species and developmental stages depending on the species. Consequently, the link between searching success and fitness is particularly close in parasitoids. It has been already shown that parasitoids adapt their behaviour and display high plasticity in their ability to adapt to different physiological and ecological conditions. However, the question of which proximate mechanisms enable this plasticity still remains largely unanswered.

The aim of this work was to study which proximate mechanism modulate the searching behaviour of parasitoid wasps by means of different experimental approaches.

Firstly a very heuristic hypothesis was tested, assuming that the searching intensity of parasitoid wasps within the microhabitat of the hosts follows a steadily decaying function. It was hypothesized that the animals respond initially with an extremely intensive searching behaviour to cues associated with the presence of hosts, which only covers a relatively small area at first, and that the gradual decay of this intensity finally leads to patch abandonment. Contacts with hosts and the border of the microhabitat served as additional modulators of searching intensity. However, it was still not possible to meet the assumptions fully, leading to the hypothesis that intermediate physiological processes are needed to modulate searching behaviour.

The subsequent studies focused on the role of the neuromodulator octopamine and its counterpart dopamine. Octopamine is assumed to have a key role in the perception of appetitive stimuli and the assessment of rewards. Dopamine, in contrast, is considered to be essential to the integration of aversive stimuli. Within a complex environment, in which optimal strategies need to be developed against the backdrop of diverse and qualitatively different stimuli, both substances seemed to be appropriate agents in the modulation of searching behaviour.

One central assumption for the subsequent studies was that the subjective assessment of hosts (and mating partners) and their related cues is sensitive to individual experiences and needs. Octopamine was supposed to modulate this assessment, while dopamine should mainly act on the assessment of potential costs. As the costs an organism is willing to invest should be relative to the expected benefits, it was expected that different kinds of investment serve as an indicator for the subjective assessment of the achieved or expected benefit. The subsequent experiments focused on the following investments: searching time, response to potential predators (stay or leave) and the response to competitors, both when competing for hosts in females and when competing for potential mates in males.

All in all, neither of the substances revealed any consistent effects with respect to the hypothesis concerning the assessment of costs and benefits in the parasitoids studied here. The impact of dopamine was restricted to slight effects on the movement pattern. This finding is consistent with results in other species. Treatment with octopamine revealed a number of effects, which in total indicate an influence of octopamine on the stress level. This result is also in line with studies on other species. Although in accordance with this octopamine significantly influences searching behaviour, the underlying mechanism is considerably more complex than initially assumed. Ultimately, it was possible to show that generalisation on the basis of a few studies and stimuli with respect to the role of octopamine in the integration of rewards is a simplification.

Zusammenfassung

Räumlich distinkt verteilte Ressourcen verlangen von Predatoren effiziente Strategien, um diese optimal auszunutzen. Auf Grund ihrer Lebensweise dienen parasitoide Insekten als Modellorganismen bei der Erforschung solcher Strategien. Adulte weibliche Parasitoide suchen für die Eiablage geeignete Wirte, bei denen es sich je nach Art um verschiedene andere Insektenarten und Lebensstadien handelt. Damit ist die Verknüpfung von Sucherfolg und Fitness bei Parasitoiden besonders eng. Es ist bereits gezeigt worden, dass Parasitoide ihr Verhalten in höchst plastischer Weise an unterschiedliche physiologische und ökologische Bedingungen anpassen. Welche proximaten Mechanismen diese Plastizität ermöglichen, ist jedoch in weiten Teilen ungeklärt.

Diese Arbeit untersucht anhand verschiedener experimenteller Ansätze welche proximaten Mechanismen das Suchverhalten parasitoider Wespen modulieren.

Zunächst wurde die heuristische Annahme überprüft, nach der die Suchintensität parasitoider Wespen innerhalb des Mikrohabitates des Wirtes einer stetig abnehmenden Funktion entspricht. Dabei wurde angenommen, dass die Tiere zunächst mit einem extrem kleinflächigen, intensiven Suchverhalten auf wirtsassoziierte Reize reagieren, und, dass das graduelle Abnehmen dieser Intensität letztlich zum Verlassen des Mikrohabitates führt. Kontakte zu Wirten und den Grenzen des Habitats dienen dabei als weitere Stellschrauben der Suchintensität. Die Annahmen konnten jedoch nicht bestätigt werden, woraus sich die Hypothese ergab, dass zwischengeschaltete physiologische Prozesse das Suchverhalten modulieren müssten.

Die weiteren Versuche fokussierten auf die Rollen des Neuromodulators Oktopamin sowie dessen Gegenspieler Dopamin. Oktopamin wird eine Schlüsselrolle in der Wahrnehmung appetitiver Reize und in der Bewertung von Belohnungen zugesprochen, Dopamin hingegen gilt als eine Art Pendant in der Verarbeitung aversiver Stimuli. In einer komplexen Umgebung, in der optimale Strategien vor dem Hintergrund diverser und qualitativ unterschiedlicher Stimuli entwickelt werden müssen, schienen beide Substanzen geeignete Agenzien für die Modulation des Suchverhaltens zu sein.

Eine zugrunde liegende Annahme für die weiteren Versuche war, dass die subjektive Bewertung von Wirten (Paarungspartnern) und deren Spuren abhängig von individuellen Erfahrungen und Bedürfnissen ist. Oktopamin, so wurde angenommen, sollte diese Bewertung modulieren, Dopamin hingegen vor allem auf die Bewertung potenzieller Kosten wirken. Da die Kosten, die ein Organismus zu investieren bereit ist, mit dem zu erwarteten Nutzen im Verhältnis stehen sollten, sollten in Kauf genommene Kosten verschiedener Art ein Indikator für die subjektive Bewertung des erreichten oder erwarteten Nutzen darstellen. In den folgenden Versuchen lag der Schwerpunkt auf folgenden Investitionen: Suchzeit, Reaktion auf einen potentiellen Predator (fliehen oder bleiben) sowie der Reaktion auf Konkurrenten, sowohl bei Weibchen in Konkurrenz um Wirte als auch bei Männchen in Konkurrenz um potentielle Paarungspartner.

Insgesamt ließen sich bei den untersuchten Parasitoiden keine konsistenten Effekte beider Substanzen in Bezug auf die Bewertung von Kosten und Nutzen zeigen. Die Wirkung des Dopamins beschränkte sich weitestgehend auf leichte Effekte in Bezug auf das Bewegungsmuster. Dieser Effekt stimmt mit Ergebnissen an anderen Organismen überein. Eine Behandlung mit Oktopamin zeigte eine Reihe von Effekten, die in ihrer Gänze auf einen positiven Einfluss dieses Stoffes auf das Stresslevel hinzeigen. Auch dieser Befund ist von anderen Organismen bereits bekannt. Zwar hat Oktopamin damit sichtlichen Einfluss auf das Suchverhalten, jedoch in einer deutlich komplexeren Form als ursprünglich angenommen. Es zeigt sich schließlich, dass ein Generalisieren auf der Basis weniger Arten und Reize in Bezug auf die Rolle des Oktopamins in der Verarbeitung von Belohnungen eine Simplifizierung ist.

1 General introduction and overview

Exploiting resources is often a complex task for animals. Resources are almost always distributed in a non-continuous fashion and are often hard to access. As such, animals have to solve the task of finding suitable places with the resources that fit their needs, and they have to allocate time and efforts across different sites where resources are found. The latter is a consequence of the diminishing return rate with ongoing exploitation by the animal itself, with the exception of the rather rare case of where constant resource replenishment exists at sites. In general, it is assumed that animals should try to maximise their intake rate (Charnov, 1976; Parker & Smith, 1990). In this light, staying too long or staying too briefly will be disadvantageous for the animal. Leaving early means giving up on a given resource when it would still be profitable to stay and leaving late translates to wasting time that could be spent elsewhere in a more profitable way. At first glance, a simple answer is offered by the marginal value theorem (Charnov, 1976), which shows that it would be optimal to leave once the instantaneous gain rate at a given site (henceforth referred to as “patch” (Hassell, 1978)) drops below the average gain rate in the habitat. Or to put it into more economic terms, the benefits of staying at that given patch are lower than leaving it. A solid assessment of the costs and benefits of any given action in terms of lifetime fitness optimisation is the heart of optimal foraging theory and, apart from the aforementioned example of patch time allocation, there are probably an infinite number of situations in which potential costs and benefits need to be offset. Animals, for instance, have to decide on whether or how much risk should be taken in order to achieve a given reward or how much energy they should invest in order to get a needed resource or even which of several necessary resources should be given priority. From this, it becomes evident that whatever decision needs to be taken requires the integration of at least some information concerning the potential outcome of each and every decision.

Over the past few decades, numerous studies have shown that observed strategies correspond at least qualitatively to the mathematical predictions for optimal behaviour. Although conducted work has covered almost every animal phylum from nematodes (Hills et al., 2004; Sawin et al., 2000) to humans (Hutchinson et al., 2008;

Louapre et al., 2010), a lot of research derives from studies on birds (e.g., (Brunner et al., 1996; Cuthill et al., 1994; Pyke, 1978; Vasquez & Kacelnik, 2000) and insects. Within the insect clade, emphasis was placed on bees (Goulson, 2000; Higginson & Houston, 2015; Roubik et al., 1995), but the polyphyletic group of the insect parasitoids (Godfray, 1994; Thiel & Hoffmeister, 2009; van Alphen et al., 2003; Wajnberg, 2006) in particular has received a lot of attention. The introduction of insect parasitoids as model organisms in the field of behavioural ecology was mostly promoted by two facts: (1) behaviour within this group is tightly linked to fitness (Godfray, 1994), caused by their biology: parasitoids forage for suitable hosts (mostly insects in different stages of development) to lay their eggs on or in. Those hosts are required for larval development and are accordingly crucial for the parasitoid’s fitness. (2) Parasitoids attack a number of insect pests and are therefore important agents within biological control in agriculture.

As already mentioned, one of the primary requirements needed for an optimal decision is the solid assessment of resource quality. Although evidently objective criteria for resource assessment exist, assessment does, however, also have strong subjective aspects (Arnott & Elwood, 2008; Thiel & Hoffmeister, 2009). A number of examples are given to illustrate this fact, although it must be noted that many more such cases exist. For instance, the physiological state and individual experiences may strongly affect the subjective value assigned to a resource: A starved animal will rank a resource which promises food higher than a sated one (Arnott & Elwood, 2008; Aw et al., 2009; Pompilio et al., 2006). An unmated sexually reproducing individual will invest more in achieving intercourse than an individual that already has mated once or more (Papadopoulos et al., 2009; Papanastasiou et al., 2013). As their life-expectancy decreases, animals will exploit reproductive sites more thoroughly, as the chances of finding other suitable sites diminish (Roitberg et al., 1993; Thiel et al., 2006; Thiel & Hoffmeister, 2004).

Numerous approaches exist for measuring subjective resource value. Firstly, time allocation can and has been extensively studied (Driessen & Bernstein, 1999; Driessen et al., 1995; Iwasa et al., 1981; Stephens, 1993; Thiel, 2011; Thiel et al., 2006; Thiel & Hoffmeister, 2004; van Alphen et al., 2003; Wajnberg, 2006; Wajnberg et al., 2013), with most of the empirical studies confirming that, first of all, most animals adapt time spent on a given resource patch to quality and, secondly, that the time spent depends on the physiological state (Wajnberg, 2006) and experience (Liu et al., 2009; Thiel, 2011; Thiel et al., 2006; Thiel & Hoffmeister, 2004; Wajnberg, 2006) of the animal.

Secondly, resource value assessment can be observed in degrees of searching intensity in the locomotory response. Basically, it is assumed that areas of a high quality should be intensively scanned, expressed by a high tortuosity and a general decreased velocity in the foraging path (Hills et al., 2004; Hills, 2006; Waage, 1978; Wajnberg et al., 2013).

Thirdly, it is possible to study the outcome of decision making processes. Being faced with conflicting stimuli, like required resources on the one hand and predator cues on the other, will force foragers to make decisions that should relate back to the assessed resource value: The higher the potential benefits of that given patch are, the more likely an animal should be to invest the potential costs of being caught (Nonacs, 2010; Roitberg et al., 2010; Stephens & Krebs, 1986).

Finally, animal behaviour in competitive situations can provide information about subjective resource assessment, since the willingness to fight and the intensity of conflicts should generally increase with estimated resource value (Arnott & Elwood, 2008).

In summary, detailed predictions can be made about what behaviour should be optimal under which conditions and numerous approaches exist to test whether or not animals behave in accordance with the expectations. However, it needs to be considered that complicated estimations about the costs and benefits of a given behaviour may incorporate large costs due to information processing and integration. It may be questionable whether tiny animals with "miniature brains" (Chittka & Skorupski, 2011; van Swinderen & Andretic, 2011) are able solve complex equations. For example: The seminal paper by Iwasa *et al.* (1981), which models optimal patch-leaving decisions for different prey distributions, contains 15 equations with another 18 in the appendix. Not even humans appear to be willing to invest such an intellectual effort just to estimate the optimal time to give up on a given task and, beyond from the purely neurological costs involved in these calculations, the time required to solve them would probably render the whole decision-making process suboptimal.

This raises the question of how optimal behaviour can be achieved for minimal internal costs. The discovery of these heuristic proximate mechanisms which allow animals to adjust behaviour quickly to various conditions is the goal of the relatively new field of optimal foraging research called “Evo-Mecho” (McNamara & Houston, 2009).

Useful mechanisms for behavioural adaptations under varying circumstances need to be fast to initiate, quickly adjustable and low in costs. Neuromodulators may be the perfect agents combining all the aforementioned needs as they might provide “one powerful means to dramatically but reversibly reconfigure the function of a sensory circuit without changing the ‘hard-wiring’” (Birmingham & Tauck, 2003). In doing so, they fulfil a vital role in regulating response thresholds (Birmingham & Tauck, 2003). *To give an example:* An increase in a response threshold makes an animal less likely to respond to a stimulus which is no longer of interest to satisfying the animal’s needs and vice versa. It is obvious that changes in response thresholds may serve as a simple mechanism for adapting, for instance, the aforementioned patch residence time to the instantaneous quality of the patch. It is conceivable that an increased response threshold towards olfactory cues emitted by the prey, so called kairomones, leads to patch abandonment.

Within the group of neuromodulators, the biogenic amine octopamine (referred to in the following as “OA”) seems to be central in modulating the response to rewards (e.g., (Birmingham & Tauck, 2003; Perry & Barron, 2013). For instance feeding honey bees an OA-containing diet increased the reporting of resource value by foragers upon returning to the nest (Barron et al., 2007). In addition, OA was also shown to lower the response threshold for sucrose (Behrends & Scheiner, 2012), suggesting that bees respond at lower doses of sucrose than the untreated control group. Furthermore, OA seems to modulate aggressiveness in a number of species: for example, selectively OA-depleted male crickets display less aggressive behaviour towards rivals (Rillich et al., 2011; Stevenson & Rillich, 2012), whereas the opposite effect is seen in a number of crustaceans (Momohara et al., 2013 and references therein). Most studies conclude that the role of OA in reward assessment is the mechanistic basis of these findings (Barron et al., 2007; Stevenson & Rillich, 2012).

Dopamine (referred to in the following as “DA”) is a prominent counterpart of OA which has been shown to modulate the response to aversive stimuli (Mizunami & Matsumoto, 2010; Schwaerzel et al., 2003; Unoki et al., 2005). As in experiments on the function of OA, most of the studies into the effects of DA were conducted using DA antagonists. They mostly revealed that DA-depleted animals failed to show the adequate response towards aversive stimuli (Mizunami & Matsumoto, 2010; Nakatani et al., 2009; Schwaerzel et al., 2003; Unoki et al., 2005) whereas DA injections induced a reduced likelihood of reacting to conditioned positive stimuli (Mercer & Menzel, 1982). Accordingly, DA and OA may be two essential protagonists in the process underlying optimal decision making that requires the integration of multiple stimuli of differing qualities. Recently it has been argued that most findings on the role of DA in stimulus response may be related to general and stimulus-specific alertness (van Swinderen & Andretic, 2011). According to the authors, one of the main functions of DA could be related to its role in shifting attention towards relevant information. In this way, the dopaminergic system functions primarily as a filter for irrelevant, distractive stimuli, whereas it leads to a rapid decline in task-specific attention if the outcome does not meet the expectations anymore. This shift in attention results in the animal's giving up. As such, the role of DA in decision-making could well exceed the integration of merely aversive stimuli and DA may be essential in the correct estimation of when leaving a given resource is better than remaining with it.

This work comprises different experiments to measure reward assessment within patchy environments using insect parasitoids on the basis of their well-known foraging behaviour (Godfray, 1994; Thiel & Hoffmeister, 2009; Wajnberg, 2006). The first study (chapter 2) deals with searching intensity in terms of locomotory response in the parasitoids *Leptopilina heterotoma* and *Venturia canescens*. According to theory on movement ecology, the most heuristic way to optimal resource exploitation can be achieved by means of a simple mechanism: Animals perceiving reliable resource cues display what is known as area-restricted searching behaviour by slowing down and performing an intensive search, characterised by a high degree of tortuosity. As time progresses, the intensity of the searching decreases, the path becomes straighter and the animal moves faster again, eventually leading to patch abandonment. Finding a resource item affects the locomotory response and may either accelerate or to some extent reverse the decreasing response depending on resource distribution (Wajnberg et al., 2013). Accordingly, if the assumptions of the theory are met, switches in searching intensity may be a straightforward measurement for instantaneous reward value assessment.

The subsequent chapters investigate whether OA and DA play a role as a control centre, shaping the reward value assessment in animals. Firstly, OA-dependent reward assessment is investigated in the parasitoid *L. heterotoma* using a patch-time allocation experiment (chapter 3). Based on the evidence that OA levels refer to the perceived value of a reward, we assumed OA-treated animals to remain on a patch of a given quality for longer than untreated conspecifics (Barron et al., 2007; Behrends & Scheiner, 2012; Mercer & Menzel, 1982; Mizunami & Matsumoto, 2010; Rillich et al., 2011; Stevenson & Rillich, 2012).

In chapter 4, OA- and DA-dependent reward assessment in the face of conflicting stimuli is investigated by mimicking the arrival of a predator while *Asobara tabida* parasitoids exploit a host patch (Roitberg et al., 2010). Again, it is hypothesized that, based on the modulation of reward assessment, OA-treated animals should be more willing to stay in the face of danger than control animals, whereas the opposite is expected of DA-treated animals, due to its role in the integration of aversive stimuli (Mercer & Menzel, 1982; Unoki et al., 2005). The fourth study (chapter 5) focuses primarily on the behaviour of female *A. tabida* wasps facing intrasexual competition assuming higher aggressiveness and a higher motivation to stay in OA-treated animals, as has been shown in, e.g., male crickets (Rillich et al., 2011; Stevenson et al., 2005; Stevenson & Rillich, 2012) and drosophilids (Dierick, 2008), and a decreased number of conflicts in DA-treated animals. Secondly, it is investigated under what circumstances density and competition affect survival. In this respect, it is assumed that competitors serve as stressors causing a decrease in longevity with density (Zurlini & Robinson, 1980) per se and that the mortality risk increases even further if the wasps have to compete for hosts and in OA-treated animals, if they are found to be more competitive. The fifth and final study (chapter 6) adapts the aforementioned study on females and competition to males of the same species.

The final part of this thesis (chapter 7) provides a brief summary of the studies conducted and discusses the findings against the background of the role of bioamines in reward value assessment in other invertebrate clades, with a particular focus on insects.

I.2 Study organisms

This work includes studies on several species of the paraphyletic group of parasitoids. Parasitoids are insects, mostly hymenopterans, which lay their eggs in (endoparasitoids) or on (ectoparasitoids) other insects. Depending on the species, they may attack different developmental stages (eggs, larvae, pupae or adults) of their hosts. Parasitoids are defined by the feeding habits of their larvae (Godfray, 1994). All parasitoid larvae feed exclusively and destructively on the tissue or haemolymph of another arthropod specimen, mostly other insects, eventually killing it. Parasitoids are considered as solitary if only a single individual can development successfully from a host and as gregarious if more than one individual may survive. Parasitoids have been repeatedly considered as being excellent model organisms for studies on optimal decision making and information use (Godfray, 1994; Thiel & Hoffmeister, 2009; Wajnberg, 2006) as they have to maximise the number of eggs successfully laid over their lifetime. Accordingly, their searching behaviour is subject to a strong selective pressure.

Figure 1-1: Female *L. heterotoma* parasitizing a *D. melanogaster* larva ©Nils Linek

Data on three species are considered in this work. The first study compares the locomotory responses of *Leptopilina heterotoma* (Thomson) (Hymenoptera: Figitidae) and *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae). *L. heterotoma* is a koinobiont, solitary endoparasitoid of the drosophilid family, whereas the asexual strain of *V. canescens* taken here attacks the larvae of phycitid moths (Lepidoptera: Pyralidae) (Driessen & Bernstein, 1999; Driessen et al., 1995; Salt, 1976; Thiel et al., 2006) including the flour moth *Ephestia kuehniella*.

L. heterotoma attacks the larvae of several *Drosophila* species (Carton et al., 1986), which in nature occur highly aggregated on rotten fruit (Hoffmeister & Rohlfs, 2001). As fermenting fruits have a high probability of being infected by drosophilid larvae (Vet et al., 1991), *L. heterotoma* first orientates towards components of the host food like yeast and alcohols (Dicke et al., 1984). If a host kairomone is detected upon arrival, the wasp displays a clear searching response, characterised by a regular pricking into the substrate with the ovipositor (van Lenteren, 1976). Once a host is hit, the animal places a single egg into it. For *L. heterotoma*, the first cue it follows is not very reliable (Vet et al., 1991), though drosophilids actively transport yeast towards their oviposition sites, yeasts also occur naturally during fermentation processes. Sensing of a host kairomone already gives a good indication of the presence of hosts and, accordingly, the motivation to search on that patch increases. Additionally, as hosts are usually aggregated, finding a host should indicate the presence of further larvae to be attacked and the searching motivation increases even further. In combination with a general decrease in searching motivation in times when no hosts are encountered, these host-related increments and the strength of the kairomone-related initial response offers a simple mechanism for optimal patch time allocation (Iwasa et al., 1981; Waage, 1979).

In contrast, *V. canescens* faces different conditions. Although the abundance of host larvae may reach pest level in insufficiently managed granaries and flour mills (Skovgard et al., 1999), patches are usually only home to low host densities (Driessen & Bernstein, 1999; Driessen et al., 1995; Thiel et al., 2006). Evidently, orientating towards potential food sources of a grain-eating larva in a granary is not much use, and consequently *V. canescens* is not attracted to uninfested grain (Driessen & Bernstein, 1999; Driessen et al., 1995; Thiel et al., 2006) but rather only to direct contact with the host's kairomone, which is a mandibular gland secretion (Corbet, 1971; Mudd & Corbet, 1982). Upon contact, the wasps display an intense searching behaviour characterized by drumming on the substrate with the antennae and rhythmic pricking of it with the ovipositor. Once a host is hit, a single egg is laid in it within a split second, because the egg is already positioned on the tip of the ovipositor.

Figure 1-2: Female *Venturia cansecens* attacking a larva of *Ephestia kuehniella* **©Hunia**

After oviposition, it takes the wasp around a minute to resume searching. This time is required to position a new egg on the ovipositor's tip (Rogers, 1972 cited in Thiel, 2004). In contrast to most drosophilid parasitoids, an egg-laying experience does not cause an increased motivation to search on a given patch; instead, the opposite is seen and each oviposition increases the chances of the animal leaving (Driessen & Bernstein, 1999; Driessen et al., 1995) but see (Waage, 1978; 1979). A decremental mechanism may be an adaptive strategy if host densities within a patch are usually low and the travel times in between two patches are short (Driessen & Bernstein, 1999). This difference in searching mechanism makes the two organisms so especially useful for the first study.

Figure 1-3: Female of *Asobara tabida* parasitizing a hidden larva of its host ©Wyss & Hoffmeister

L. heterotoma is also used for the second study, whereas the remaining experiments were conducted using another koinobiont, solitary endoparasitoid of the *Drosophila* species, namely *Asobara tabida* (Nees) (Hymenoptera: Braconidae). *A. tabida* orientates itself similarly to *L. heterotoma* (Vet & van Opzeeland, 1984). However, the searching behaviour on patches differs from the one described above (Vet & van Alphen, 1985). Upon arrival on the patch, *A. tabida* females slow down and alternate between standing and walking at short intervals. When standing still, the wasps localize the position of the host larvae via vibrations caused by larval movement (vibrotaxis) (Vet & van Alphen, 1985). Once located, the wasp quickly moves towards the host, remains motionless for a short time and then probes into the host. If the host is accepted, the ovipositor is inserted even deeper and the egg is laid, after which the wasp resumes searching.

Compared to *L. heterotoma*, *A. tabida* is the more active species. They initiate flight activity more readily and walk faster overall. As a feeding approach was taken in the experiments, manipulating the aminergic balance, it is assumed that individuals of *A. tabida* will be more likely to feed more often compared with *L. heterotoma* causing less variability in the data concerning the drug-treatment effect as a higher overall intake rate is expected.

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Chapter 2

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Analysis of walking behaviour as a correlate of motivation in two parasitic wasps

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Contributions: Conceived and designed the study: JU & TSH. Data collection: This work builds upon data originally collected by Christine Diekhoff, Heike Schmitz, Ulf Tölch and me for our Diploma-theses and by Pablo Gonzales Torres as part of his research project. Analyzed the data: JU & TSH. Wrote the paper: JU & TSH.

(to be submitted to Movement Ecology)

2.1 Abstract:

It has recently been demonstrated that correlated random walks of foragers could result in patch exploitation rates in line with the marginal value theorem. Mainly locomotory responses to prey encounter and upon arrival at the patch edge in combination with a general decay in searching intensity may be sufficient to adaptively allocate patch times. This study tests some of the model's assumptions in two different species of parasitic wasps. Based on host distribution, the parasitoid *Leptopilina heterotoma* was expected to re-intensify its searching effort after host encounters, whereas the opposite should be the case for *Venturia canescens*. Although searching intensity wanes over time in general, the results of this study reveal only minor locomotory responses to prey encounter in both species. In contrast, crossing the borders of the patch affected the walking path more strongly, especially in *L. heterotoma*. We hypothesize that this finding may point to the framework of reward and reward prediction. The more reliably a given surrounding predicts the presence of prey, the less pronounced the actual response to the prey itself should be. Instead, the stimulus predicting the availability of a prey (in this case the host-related kairomones) should trigger the strength of the motivational response.

2.2 Introduction

The combination of ultimate and proximate branches of ecological research has been claimed repeatedly (Nathan et al., 2008; Pierre, 2011) and has just recently been named "Evo-Mecho" (McNamara & Houston, 2009), stressing the necessity of merging proximate mechanisms (how?) and ultimate causes (why?) of behaviour.

Two highly related though more or less independently evolved fields of behavioural ecology are movement ecology and foraging ecology. The former offers a variety of more or less heuristic models describing observed walking paths within heterogeneous environments by means of mathematical functions (Nathan et al., 2008; Turchin, 1998). In contrast, foraging ecologists focus on the ultimate causes (the fitness consequences) of different searching strategies (Stephens & Krebs, 1986).

Just recently a model has been published proving the applicability of common movement models in respect to optimal patch time allocation (Wajnberg et al., 2013). The model assumes: (1) a decaying searching intensity over time, expressed as an increase in velocity and a decrease in turn angle, leading to an overall faster displacement over time. (2) A probability of "bouncing back" upon encountering the patch edge, leading the animal to head back into the centre of the patch. In this respect, the probability of bouncing back decreases the more often the edge is encountered. (3) An impact of host encounters on locomotory behaviour, leading to a re-intensification if prey is distributed in an aggregated manner and to a further decrease in case of regularly distributed prey items. (4) An increase in the probability of bouncing back as a cause of prey encounter. The combination of those four parameters leads to patch times well in line with the marginal value theorem (MVT) (Charnov, 1976), whereby the 1st and the 3rd variable have the strongest impact on optimal patch time allocation.

In this way, the model builds up on the mechanism originally proposed by Waage (1979), which was subsequently adapted by numerous authors. All of them share the combined effects of a general time dependent decay and prey encounter dependent effects on “motivation” (Lefebvre et al., 2007; Louapre et al., 2011; Pierre, 2011), “responsiveness to the patch edge” (Waage, 1979), “tendency to stay” (or to leave) (Driessen & Bernstein, 1999) or “estimated number of remaining prey” (Iwasa et al., 1981).

The effects of prey density, distribution and related cues on patch time allocation have been thoroughly tested in insect parasitoids especially by the means of Cox proportional hazards analysis (see van Alphen et al., 2003; Wajnberg, 2006 for reviews) and in general patch-time increases with intensity of host related cues and host abundance. Furthermore, in animals assumed to be adapted to clumped host distributions, each host encounter seems to decrease the leaving tendency, like in *Leptopilina heterotoma* (Thomson) (Hymenoptera: Figitidae) (Fauchald & Tveraa, 2003; Haccou et al., 1991; Rosenheim, 1996), whereas the opposite is the case for animals adapted to more regularly distributed hosts like *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) (Driessen & Bernstein, 1999; Driessen et al., 1995). However, it is difficult to state clearly the direct effect of the different on-patch cues on the stated “searching motivation”, even though Cox proportional hazards analysis tend to do so. Yet even Cox analysis does not solve the problem of causality and correlation (Pierre, 2011; Thiel & Hoffmeister, 2009). Detailed analysis of the effect of different events on the movement pattern may help to elucidate the causal consequences of different patch quality related experiences, which may become apparently clear by Hills’ description of animal movement (Hills, 2006) and its relatedness to the internal state: Animal foraging can in general be described as area-restricted search (ARS). ARS is a ubiquitous pattern in moving organisms ranging from bacteria to humans and is adaptive in case of clumped distributed resources. Encountering a patch associated with a resource elicits an arrestment response in the forager. The animal slows down and switches to a more intense searching mode characterised by high tortuosity and low velocity (Waage, 1978). As the animal exploits the patch, it will have a diminishing encounter rate. This should be reflected in a behavioural change; the search becomes less intensive, but covers more space (Hills, 2006). Finally, the animal leaves the patch. Hills (2006) points out that "*ARS is a mechanism for giving up when the environment no longer meets expectations. In this respect, it may be very closely related to basic neural mechanisms such as habituation and sensitization*".

Interestingly, detailed analyses of locomotory responses to different experiences are even rare from the side of movement ecologists, as most studies dealing with exploitation dependent movement adaptations are either theoretical or fail to disentangle proximate changes in behaviour from ecological changes in resource availability, as they mostly follow populations in the field (Bazazi et al., 2012). For example: a gradual decrease in tortuosity may simply mirror the increasing distances inbetween the prey items and not be related to an adaptive proximate searching strategy.

The goal of this study is to test the assumed decrease of intensity of searching behaviour over time and the effects of oviposition experiences on the walking pattern in two different parasitoid species *(V. canescens* and *L. heterotoma*). Both are solitary parasitoids, but differ in respect to their proposed searching mechanism. Whereas *V. canescens* is known to have a decremental searching mechanism (Driessen & Bernstein, 1999; Driessen et al., 1995), meaning that each host encounter decreases the tendency to stay, *L. heterotoma* has an incremental mechanism (Fauchald & Tveraa, 2003; Haccou et al., 1991; Rosenheim, 1996), typical of animals hunting for aggregated prey. Bouncing back behaviour is hard to measure, as we do not know anything about the reactive distance, meaning the distance from the animal to the patch border when the animal perceives the information that it has reached the end of the patch. Instead we analysed the impact of excursions from the patch. Most parasitoids leave the patch temporarily during their search, but then turn back. For both species, it is known that re-encountering the patch has a strong effect on the remaining patch time (Bernstein & Driessen, 1996; Haccou et al., 1991) decreasing the tendency to stay in both species, which would correlate with the decreasing probability of bouncing back in the movement model (Wajnberg et al., 2013).

In the first step, the proposed searching mechanism (incremental or decremental) was verified based on Cox regression. Subsequently, locomotory behaviour, in the forms of velocity and turn angle, was analysed as a function of time as well as of excursions and ovipositions. According to the movement model developed by Wajnberg et al. (Wajnberg et al., 2013) and the general description of an ARS (Hills, 2006), we expected a generally waning searching intensity, meaning an increase in velocity with a corresponding decrease in tortuosity over time in both species, while ovipositions should lead to a re-intensification in *L. heterotoma* and to a further decrease in *V. canescens*. Returning to the patch was assumed to come along with a decreased searching intensity in both species (according to Bernstein & Driessen, 1996; Haccou et al., 1991).

2.3 Material and Methods

Three sets of experiments in total were conducted on two different solitary endoparasitoid species, *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae), and *Leptopilina heterotoma* (Thomson) (Hymenoptera: Eucoilidae). The first set of experiments was done on *V. canescens* and served to analyse the effects of oviposition experience and excursions on the leaving tendency as well as locomotory behaviour. With *L. heterotoma*, two independent experiments were conducted. The first was taken to run the Cox analysis, but was deficient for the analysis of locomotory behaviour as handling of the larvae by the experimenter caused disturbances. Consequently, a second experiment was conducted to analyse the effects of oviposition and excursions on locomotory response.

The parthenogenetic (thelytokous) type *of V. canescens* used here is found frequently in granaries and mills searching for moth larvae. Host abundance in this species is usually low but variable (Driessen et al., 1995; Waage, 1979) and may even raise quickly to enormous numbers (Thiel et al., 2006)*.* *L. heterotoma* is a parasitoid of several *Drosophila* species (Carton et al., 1986), which occur ephemerally and highly aggregated (Hoffmeister & Rohlfs, 2001).

2.3.1 *Venturia canescens*

Two-day-old naïve thelytokous wasps were used. The wasps belonged to two different lines, originating from Antibes, France, and Valence, France, where they were caught roughly five years before the experiments took place. They had been reared in the lab ever since, using the flour moth *Ephestia kuehniella* as a host.

The experimental arena of approximately 12 cm in diameter was composed of fresh semolina and experimental patches of approximately 4 cm in diameter sunk flush to the surface of the arena (as described in Thiel et al., 2006). Uninfested semolina *per se* is not attractive for *V. canescens* and usually elicits no arrestment response (Driessen et al., 1995). Patches were prepared by letting four host larvae feed in semolina for two days. While feeding, the larvae leave some secretions from the mandibular gland, which work, as they are used by the wasp to locate the hosts (Corbet, 1971), as so called "kairomones". Just moments before the experiment took place, those hosts were removed, and a mini-patch constructed from the lid of an Eppendorf® cap containing either no host (empty, N=12) or a single healthy host (host, N=13) was placed in the experimental kairomone patch.

Behaviour was scored using EthoVision (Noldus, Wageningen, Netherlands). The program automatically scores velocity and turn angle based on a 0.48 pictures/s frame rate and gives the position of the searching animal based on manually defined zones (on patch or off patch). The turn angle was measured as a degree per unit of time, in this case 0.48 s. A trial was terminated if the wasp either walked off the experimental arena or flew off the patch.

Despite the position and the movement parameters, the following behaviours were manually scored: (1) searching, when the animal was using its antennae to palpate the patch; (2) probing, when the animals drilled into the substrate with the ovipositor; (3) "cocking", describes the characteristic process of placing an egg on the tip of the ovipositor (Rogers, 1972); (4) preening.

2.3.2 *Leptopilina heterotoma*

Patch-residence times and movement parameters were analysed based on two sets of experiments both using the same population of *L. heterotoma,* originating from

Wageningen, Netherlands, and reared in the lab since 1995 on a native host species, *Drosophila melanogaster*.

Females were kept isolated from hatching until the experiment with two males and *ad lib* honey in small glass vials. One day (24 ± 2 h) prior to an experiment and again directly before the experiment the female wasps were allowed to search a pre-experimental patch with 16 host larvae for 20 minutes, as naïve females of this species are known to have only limited parasitization success (Samson-Boshuizen et al., 1973) and pre-experience significantly reduces the time needed to enter the patch (Perez-Maluf et al., 2008). It was ensured that all females had parasitized at least one host during this time. Females which did not display searching behaviour at all during the allotted time period were discarded. After their second pre-experience, the wasps were stored in a gelatine capsule for five minutes before they were introduced into the experimental arena.

Experimental patches consisted of 9-cm Petri dishes lined with agar and a yeast patch 2 cm in diameter in the middle. Eight 2nd instar larvae were allowed to move freely on the patch for 15 minutes. During feeding, the larvae, similar to *V. canescens,* leave chemical traces (Dicke et al., 1985). These kairomones lead to the characteristic searching behaviour upon contact in *L. heterotoma*.

In the experiment testing the patch residence time, all the hosts were removed from the patch directly before the wasp was introduced. After 140 seconds of free search, a host was placed near to the wasp using a fine paint brush in half of the experiments (N=55). In another 43 replicates serving as the control, the paint brush was also introduced, but without a larva, to ensure that no disturbance-specific differences would occur. This procedure made it possible to minimise variance caused by random differences in host finding, but, however, caused disturbances in the scoring of the behaviour. Accordingly, another set of experiments was conducted in which, in order to minimise disturbances caused by the brush, either two or no host remained on the patch. We decided to take two hosts in order to enhance the chance of finding a host before deciding to leave.

As in *V. canescens,* behaviour was scored using EthoVision (Noldus, Wageningen, Netherlands).

2.3.3 Statistical Analysis

Patch-residence time and tendency to stay

Patch residence time was defined as the time interval lasting from first entering of the patch until the time the patch was left for more than thirty seconds.

The impact of ovipositions and excursions on the tendency to stay was analysed using Cox proportional hazards, (Hoffmeister et al., 2006; Therneau, 2012) in R.2.15.2 (R-Core-Team, 2014).

The best model was found based on the AIC and the final models were tested for proportionality of the time-dependent effects based on Schoenfeldt-residuals (Therneau, 2012). Testing for proportionality is necessary as it may well be possible that the impact of an event on the tendency to stay changes over time. If the proportionality criterion is not violated, it can be assumed that the effect of a given event is not time-dependent. Furthermore, the effect of patch quality on the number of excursions as well as of their first initiation was analysed by the means of GLM (Fox & Weisberg, 2011).

Walking pattern

The effect of searching time as well as of ovipositions and excursions on locomotory behaviour was tested. Therefore, first the mean velocity and the mean turn angle of the last 10 seconds before an event and the first 10 seconds after resumption of searching behaviour were calculated. To ensure that differences before and after an event were actually induced by the event itself and not due to time effects, walking behaviour was additionally compared to corresponding time intervals without any event. As such, random intervals were generated out of the data distribution of the real events. For that purpose, data distribution of the real events needed to be normally distributed. This assumption was only met for the duration of ovipositions while the times of initiation of excursions as well as of ovipositions and the duration of excursions were right-skewed and had to be square root transformed before corresponding random intervals could be generated. Normality was tested using Shapiro-Wilk tests in R 2.15.2. Matching of real and random intervals was ensured by t-tests. The data created that way determined the last value before and the first value after an “event”. As for the real events, the mean velocity and the turn angle were calculated for the 10 previous and the 10 subsequent seconds.

Ovipositions were defined as successful if the host larvae were pierced for more than 16 s in *L. heterotoma* (Haccou et al., 1991) or if the probing was succeeded by cocking behaviour in case of *V. canescens* (Rogers 1972). Excursions shorter than 2 s were omitted from the analysis. Finally, intervals were cut off if they were interrupted by other events, which in some cases resulted in shorter intervals, but took into account that experience did not change within an interval except for the time spent searching.

Statistical analysis was conducted using the lme4 package in R.2.15.2 (R-Core-Team, 2014) for linear mixed models (Bates et al., 2013) with "wasp" as the random factor. Including the random term is necessary, as each wasp contributes to the analysis with a number of data points, which, accordingly, cannot be taken as independent anymore. Explanatory variables were selected using backward elimination of non-significant effects (Nelder & Wedderburn, 1972; Wajnberg & Haccou, 2008).

The distribution of residuals was analysed visually. Most explanatory variables were right skewed and accordingly log or square root transformed. The appropriate transformation was selected based on Shapiro-Wilk tests for normality.

The effect of ovipositions was only analysed within the data subsets with host encounters; for the analysis of the excursions, the complete datasets were taken and patch quality served as an additional explanatory variable.

2.4 Results

2.4.1 Patch leaving and movement in Venturia canescens

2.4.1.1. Patch Residence Time

Wasps had on average 1.77 ±0.28 ovipositions, indicating a high tendency to superparasitize. Out of 13 wasps with ovipositions, seven only laid a single egg, three placed two eggs in the offered host, in two wasps three cockings were observed and in one individual even four such events occurred. However, for the effect on the

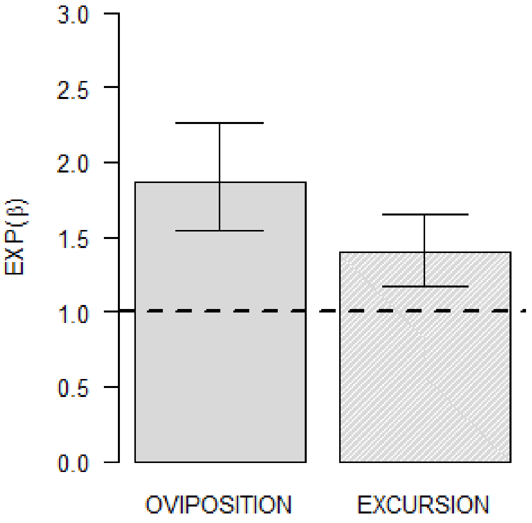


Figure 2-1: Effects of ovipositions (left bar) and excursions (right bar) on the tendency to stay on the host patch of *Venturia canescens* based on the proportional hazard (exp(beta)) of a Cox regression analysis. Values for exp(β) > 1 indicate a decrease in the tendency to stay. The overall significance of the model based on Wald test: = 16.89 on 2 df = 1, p=0.0002146.

tendency to stay, it made no difference whether it was the first or a subsequent oviposition, as the effect was proportional over time (Analysis of Schoenfeldt residuals: ovipositions: rho=0.14, χ^2=0.52, P=0.47) and each oviposition had the same strong decremental effect on the tendency to stay (N=27, exp(β)= 1.87, se(β)=0.19, Z= 3.29 P= 0.001, see Fig. 2-1).

The first excursion occurred on average after 344.22s ± 88.9s if no host was on the patch and already after 204.6s ±72.27s if a host was present. Yet, this difference was not significant (GLM with Gamma and log-link function: patch quality: χ^2_{25}= 1.3, df=1, P= 0.25, see figure 2-2a). The total number of excursions was with 2.75 ± 0.45 on an empty patch and 2.22 ±0.4 on a patch with host again independent of patch quality (GLM with Poisson-distribution and log-link function: χ^2_{25}= 0.57, df=1, p= 0.45, see Figure 2-2b).

Nevertheless, each excursion seemed to decrease the tendency to stay, and, even though this effect is slightly insignificant (N=25, exp(β)= 1.39, se(β)= 0.17, Z= 1.94 P=0.05, see Figure 2-1) the excursion effect should, based on the AIC, remain in the model (AIC without excursion: 127.36, with excursion: 125.68). As for the ovipositions, the excursion effect does not depend on time, but in contrast is stable, meaning that an excursion occurring later during the search has the same effect on the tendency to stay as an earlier one (Analysis of Schoenfeldt residuals: excursions: rho=-0.06, χ^2=0.10, P=0.76).

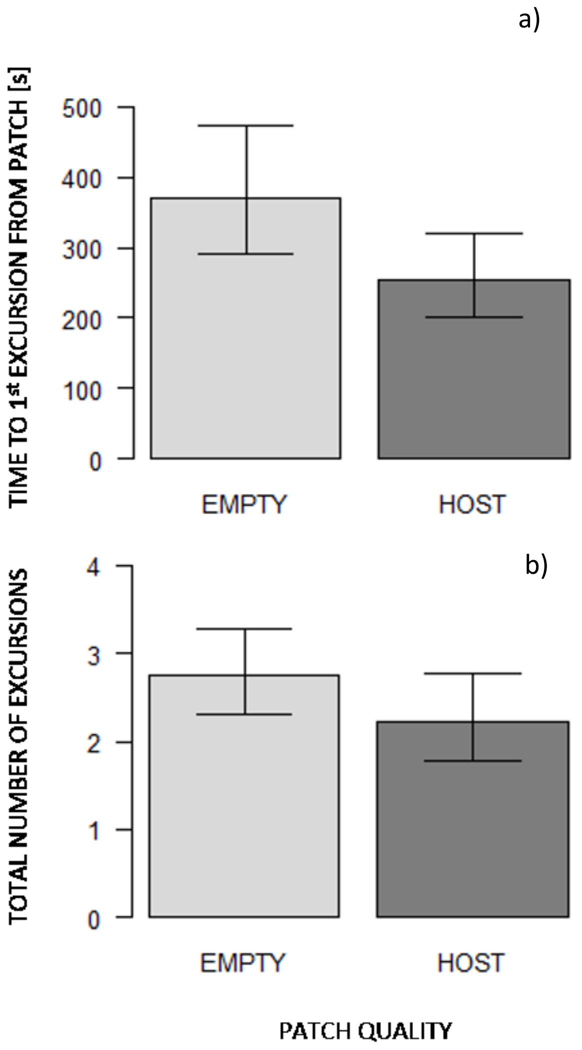


Figure 2-2: Searching behaviour of Venturia canescens on host patches a) Time of initiation of the first excursion from the patch and b) total number of excursions on either empty patches or patches containing a single host larvae in *V. canescens.* Shown values are predicted means and standard errors taken from the GLM-analysis.

Furthermore, the excursion effect does not depend on oviposition experience as the interaction of oviposition and excursion is not significant (β=-0.21, exp(β)=0.81, se(β)= 0.19, z=-1.08, P= 0.28).

The results of the Cox regression analysis confirmed the expectations and other empirical results with regard to the decremental searching mechanism of *V. canescens* (Driessen & Bernstein, 1999; Driessen et al., 1995) but see (Amat et al., 2006). Furthermore, they indicate the role of excursions, which may incorporate a number of important information for the predator like the size of the patch, nature of the environment and minimal distance to the next patch. In contradiction to the model (Wajnberg et al., 2013) and to the assumptions by Waage (1978; 1979) ovipositions did not seem to affect the effect of excursions as the interaction of both terms was not significant. However, we can still assume based on the heuristic mechanism described above that both ovipositions as well as excursions would lead to a waning searching intensity, which should be expressed by an increase in walking speed and a correlating decrease in tortuosity leading to a straighter walking path.

2.4.1.2 Effects of ovipositions and excursions on walking behaviour in *V. canescens*

Effects of ovipositions on the walking path

Table 2-1: Effects of ovipositions on walking behaviour in *V. canescens*, based on Linear Mixed Effect Models. All possible interactions were tested (only shown if significant).

			Velocity [cm/s] (GLMM with Gaussian distribution and inverse-link function)		**Turn Angle [°]** (GLMM with Gaussian distribution and log-link function)	
	N	**df**	**χ^2**	**p**	**χ^2**	**p**
Event quality (real or random)	13/92	1	0.56	0.46	5.29	**0.02**
Interval: Before or after the event	13/92	1	2.88	0.09	0.18	0.67
Sqrt(Time)	13/92	1	7.49	**0.01**	5.50	**0.02**
Event quality * nterval	13/92	1	0.12	0.73	0.16	0.69
Event quality *sqrt(Time)	13/92	1	0.89	0.34	0.51	0.48
Sqrt(Time) * Interval	13/92	1	5.40	**0.02**	< 0.01	0.97
Sqrt(Time) * Event quality * Interval	13/92	1	4.63	**0.03**	0.01	0.94

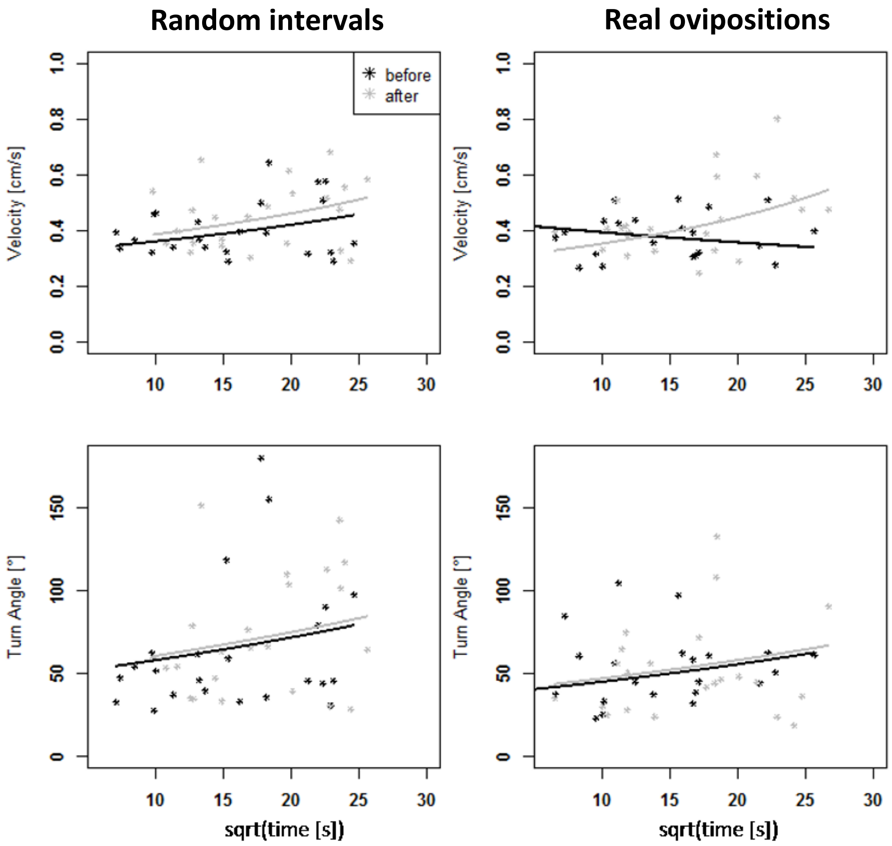


Figure 2-3: Changes in walking behaviour of *V. canescens* within the 10 s before (black) and the 10s after (grey) an oviposition as a function of current patch time [s]. To achieve normality time on patch was square root transformed prior to analysis. Upper graphs refer to the changes in the walking velocity [cm/s], lower graphs to the changes of the turn angle [°] of the search path, measured as the change in direction per frame of the videographed search path. The left column refers to randomly chosen time intervals without an oviposition event, the right column to real ovipositions. Lines refer to the line of best fit based on GLMM (see table 2-1).

However, the results are not that clear cut (see Table 2-1, see Fig. 2-3). Velocity generally increases over time, with one exception being the interval before an oviposition. Animals walk faster in the interval after a (pseudo-)event compared to the one before. Though this effect is found in the case of a real oviposition event as well as in the random intervals, it is more expressed in the context of real ovipositions indicating a combined effect of general time differences (the second interval is always after the first) and oviposition experience. In contrast to initial expectations, the turn angle increases over time, again irrespective of the quality of the event, though tortuosity is consistently higher within the randomly chosen intervals.

Effects of excursions on the walking path on the patch

Velocity is most affected by the time the animals have spent already on the patch. Irrespective of whether excursions really took place or not, all animals became faster over time (see Table 2-2, see Figure 2-4). Additionally, the model indicates an effect of the event quality, and prior and after an excursion *V. canescens* females walk slightly faster (0.018 cm/s) than in context with random intervals. Finally, the slope over time is slightly steeper within the intervals after a (pseudo-) event. However, as this is retrieved irrespective of event quality, this is most likely due to time effects. Interestingly, whether a host is present or not does not affect velocity at all either per se or in any interaction.

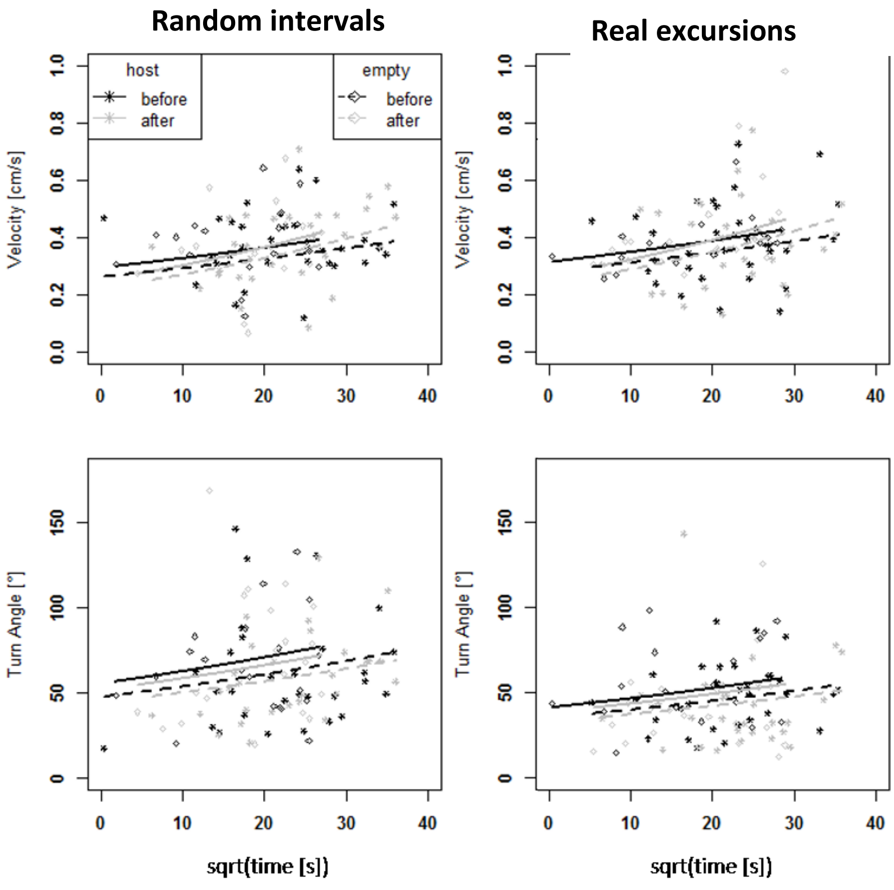
In contrast, tortuosity differs significantly depending on patch quality and animals searching in the presence of a host consistently have a higher turn angle (see Table 2-2, see Figure 2-4). Furthermore, the walking path is straighter in the context of real excursions than in randomly chosen intervals. However, no additional impact of returning to the patch is found as interval is not significant either alone or in any interaction. Consistent with the analysis of the ovipositions and again in contradiction to the predictions, the turn angle again increases over time.

Table 2-2: Effects of excursions on walking behaviour in *V. canescens* based on Generalized Linear Mixed Effect Models, tested were all possible interactions up to three-way interactions (only shown if significant). [1]The model including those variables explains the data best based on AIC.

			Velocity [cm/s] (GLMM with Gaussian distribution and log-link function)		**Turn Angle [°]** (GLMM with Gaussian distribution and log-link function)	
	N	**df**	**χ^2**	**p**	**χ^2**	**P**
Patch quality (empty or with host)	26/196	1	0.57	0.45	4.58	**0.03**
Event quality (real or random)	26/196	1	3.1	**0.08**[1]	17.34	**3.13e-05**
Interval: Before or after the event	26/196	1	0.38	0.54	1.00	0.32
Sqrt(Time)	26/196	1	19.05	**1.28e-05**	6.72	**0.01**

Table 2-2 (continued):

Interval * Event quality	26/196	1	0.82	0.36	2.82	0.09
Interval * sqrt(Time)	26/196	1	3.49	**0.06**[1]	0.81	0.37
Interval * Patch quality	26/196	1	1.3	0.25	< 0.01	0.97
Event quality * sqrt(Time)	26/196	1	0.7	0.4	0.02	0.88
Event quality* Patch quality	26/196	1	1.21	0.27	0.01	0.92
Sqrt(Time) * Patch quality	26/196	1	2.50	0.11	0.07	0.79


Random intervals

Real excursions

Figure 2-4: Changes in walking behaviour of *V. canescens* within the 10 s before (black) and the 10s after (grey) an excursion as a function of current patch time [s] in *V. canescens*. To achieve normality time on patch was square root transformed prior to analysis. Upper graphs refer to the changes in walking velocity [cm/s], lower graphs to the changes of the turn angle [°] of the search path, measured as the change in direction per frame of the videographed search path. The left column refers to randomly chosen time intervals without an excursion, the right column to real excursions. Lines refer to the line of best fit based on GLMM (see table 2-2). Dashed lines refer to wasps searching on an empty patch, solid lines to wasps with host encounter.

2.4.2 Patch leaving and movement in *Leptopilina heterotoma*

2.4.2.1 Patch Residence Time

Superparasitism did not occur in *L. heterotoma*. On average it took the wasps 200.62 s ± 11.01s to attack the offered host successfully (N=53).

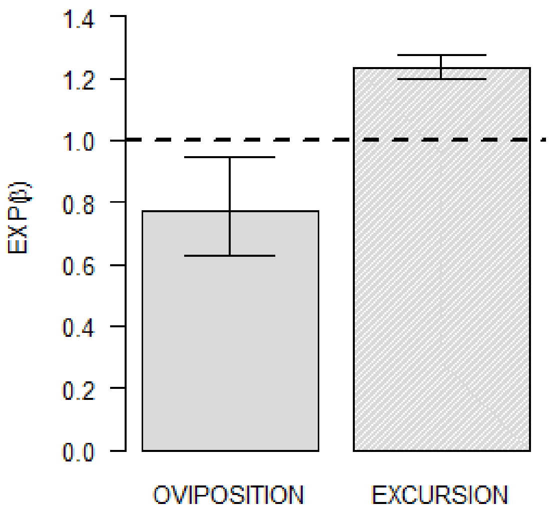


Figure 2-5: Effects of oviposition (left bar) and excursions (right bar) on the tendency to stay and continue searching in a host patch in *L. heterotoma* based on the proportional hazard (exp(beta)) of a Cox regression analysis. Values for exp(BETA) > 1 indicate a decrease in the tendency to stay. The incremental effect of oviposition is not significant (p= 0.211) nor kept in the model based on AIC, though shown to illustrate the estimated exp(β). The overall significance of the minimal adequate model (without oviposition) based on Wald test: = 67.69, df=3, p=1.332e-14. The decremental effect of excursions is highly significant (P=2.51e-10).

Even though wasps finding a host stay with 781.99±6.37 s longer on patch overall than those ones searching on an empty patch (608.56±6.37s, GLM with Gamma distribution: χ^2_{98}=10.64, df=1, p= 0.001) this effect seems to be not uniquely due to the oviposition experience per se, as Cox regression analysis fails to detect a significant incremental effect on the tendency to stay (β= -0.26, exp(β)= 0.77, se(β)=0.21, z= -1.25, P= 0.21, see Fig. 2-5) in the case of ovipositions. As in *V. canescens,* ovipositions again did not alter the effect of excursions on the tendency to stay (β=0.07, exp(β)= 1.08, se(β)=0.06, z=1.15, P= 0.25).

If the patch was empty, wasps left it for the first time on average after 512.65 s (N=43). This was significantly earlier compared with wasps searching on a patch with hosts present (652.23 s, N=55, GLM with Gamma distribution: χ^2_{98}=40.97, df=1, p=1.55e-10, see Figure 2-6). However, patch quality seemed to have no effect on the total number of excursions GLM with poisson distribution (χ^2_{98}=0.10, df=1, p=0.75) and wasps left the patch irrespective of its quality approximately 4 times (see Figure 2-6) before they finally left it, leading to an overall higher frequency of excursions within those wasps without oviposition success.

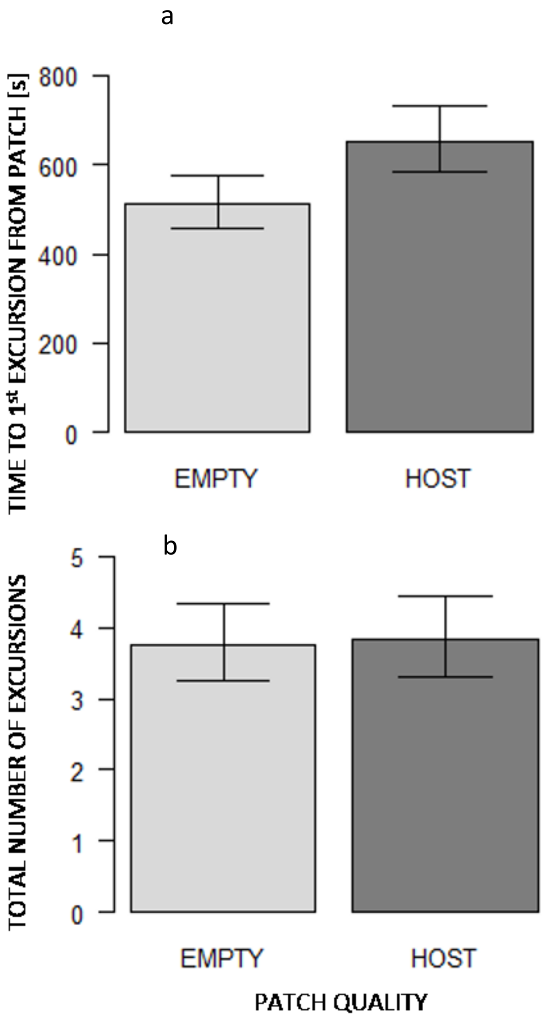


Figure 2-6: Searching behaviour of *L. heterotoma* on host patches. a) Time of initiation of the first excursion from the patch and b) total number of excursions on either empty patches or patches containing a single host larva in *L. heterotoma*. Shown values are predicted means and standard errors taken from the GLM-analysis.

Each excursion decreased the tendency to stay by more than 20% (β=0.21, exp(β)=1.24, se(β)=0.03, z=6.35, P=2.10e-10, see Fig. 2-5). As in *V. canescens*, this decremental effect was irrespective of time, as the analysis of Schoenfeldt residuals did not reveal any disproportionalities (rho=-0.02, χ^2=0.07, P=0.87).

The results of the Cox regression analysis only partly confirmed the expectations and other empirical results with regard to the incremental searching mechanism of *L. heterotoma*. Although the effect of excursions corresponds to other data on *L. heterotoma* (Haccou et al., 1991), the effect of ovipositions is not significant. Yet, the estimated exp(β) is similar to the one in Haccou et al. (1991). As in *V. canescens*, no effect of ovipositions on the effect on excursion was determined.

2.4.2.2 Effects of ovipositions and excursions on walking behaviour in *L. heterotoma*

The inspection of the data-distribution indicated that velocity and turn angle might follow a hyperbolic progression. Accordingly, in addition to the square root of time, the variable was added in its second order as well.

Effects of ovipositions on the walking path

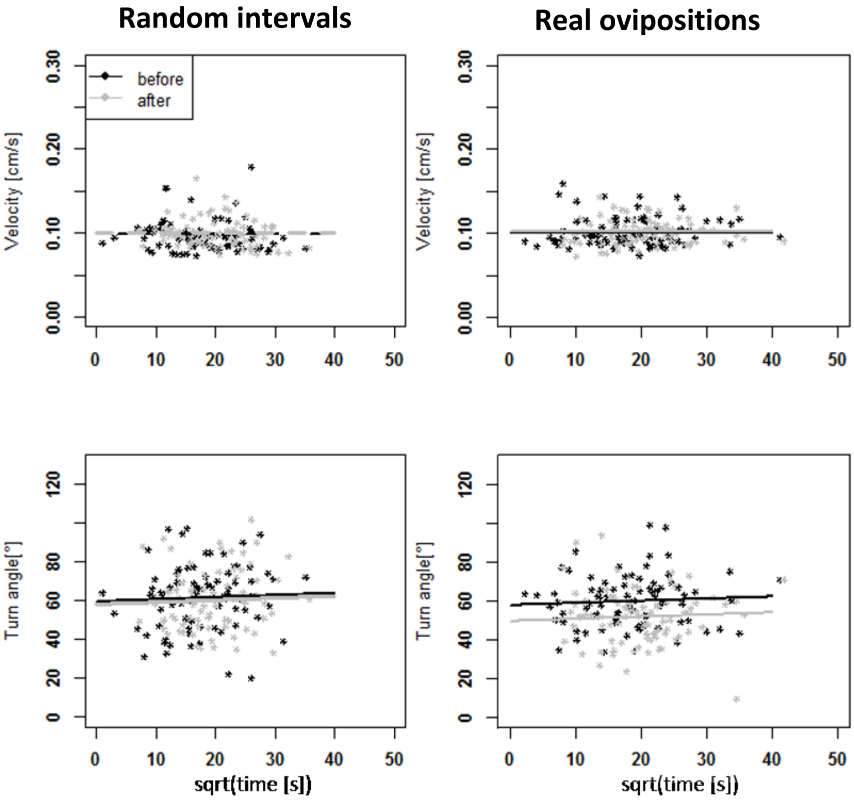


Figure 2-7: Changes in walking behaviour of *L. heterotoma* within the 10s before (black) and the 10s after (grey) an oviposition as a function of current patch time [s]. To achieve normality time on patch was square root transformed prior to analysis. Upper graphs refer to the changes in the walking velocity [cm/s], lower graphs to the changes of the turn angle [°], of the search path, measured as the change in direction per frame of the videographed search path. The left column refers to randomly chosen time intervals without an oviposition event, the right column to real ovipositions. Lines refer to the line of best fit based on GLMM for velocity and LMM for turn angle respectively (see Table 2-3).

The average walking speed in *L. heterotoma* is not affected by any of the explanatory variables and wasps move steadily at 0.0998 ±0.0009 cm/s irrespective of the patch quality, event quality, time or interval (see Table 2-3, Figure: 2-7).

In contrast, the turn angle seems to be more vulnerable to changes, which is mainly due to the effect of ovipositions, whereas in the analysis of velocity no general trend over time is recorded (see Table 2-3, Figure 2-7). However, an oviposition experience leads to a decrease in tortuosity (see Figure 2-7), which is exactly the opposite of what was expected. As in the analysis of velocity, no time-dependent effects were detected.

Table 2-3: Effects of oviposition experience on walking behaviour in *L. heterotoma* based on Generalized Linear Mixed Effect Models, tested were all possible interactions (only shown if significant)

			Velocity [cm/s] (GLMM with Gaussian distribution and log-link function)		**Turn Angle [°]** (LMM)	
	N	**df**	**χ^2**	**p**	**χ^2**	**p**
Event quality (real or random)	42/328	1	0.53	0.47	10.23	**0.001**
Interval (before or after the event)	42/328	1	0.42	0.52	8.15	**0.004**
Sqrt(Time)	42/328	1	0.01	0.93	0.75	0.39
Sqrt(Time)^2			0.02	0.89	0.05	0.83
Event quality * Interval	42/328	1	1.28	0.26	4.53	**0.03**

Effects of excursions on the walking path on patch

Analysis of excursions reveals the effect of a number of variables and their interactions on the walking pattern (see Table 2-4, Figure 2-8).

First of all, animals searching successfully (solid lines) have a lower velocity compared to the ones searching on an empty patch (dashed lines). This effect remains consistent over time. Secondly, velocity is higher in context with an excursion compared to randomly chosen intervals. However, whereas in animals being confronted with an empty patch re-entering of the patch leads to a strong re-intensification in searching behaviour, expressed as a decrease in velocity, this interval effect is not recorded in animals experiencing host encounter. The development of velocity over time is best described by a hyperbolic curve with a peak at medium times.

Table 2-4: Effect of excursions on walking behaviour in *L. heterotoma* based on Generalized Linear Mixed Effect Models, tested were all possible interactions up to three-way interactions (only shown if significant)

			Velocity [cm/s] (GLMM with Gaussian distribution and log-link function)		**Turn Angle [°]** (GLMM with Gaussian distribution and log-link function)	
	N	**df**	**χ^2**	**P**	**χ^2**	**p**
Patch quality (empty or with host)	73 / 1117	1	11.68	**< 0.001**	63.90	**1.31e-15**
Event quality (real or random)	73 / 1117	1	97.48	**< 2.2e-16**	132.06	**< 2.2e-16**
Interval (before or after the event)	73 / 1117	1	12.73	**< 0.001**	0.29	0.59
Sqrt(time)	73 / 1117	1	9.27	**< 0.01**	30.74	**2.95e-08**
Sqrt(time)^2			9.83	**< 0.01**	10.98	**< 0.001**
Event quality * Interval	73 / 1117	1	7.91	**< 0.01**	18.54	**1.67e-05**
Event quality * Patch quality	73 / 1117	1	1.12	0.29	10.27	**< 0.01**
Interval *Patch quality	73 / 1117	1	16.51	**4.84e-05**	n.s.	n.s.
Interval* sqrt(time)^2	73 / 1117	1	n.s.	n.s.	9.24	**< 0.01**
Patch quality * sqrt(time)^2	73 / 1117	1	n.s.	n.s.	13.06	**< 0.001**
Event quality * Interval * Patch quality	73 / 1117	1	11.29	**< 0.001**	n.s	n.s.
Event quality * sqrt(time)^2 * Patch quality	73/1117	1	n.s.	n.s.	15.46	**8.44e-05**
Event quality * Interval * sqrt(time)^2	73/1117	1	n.s	n.s	4.34	**0.04**

The turn angle is best described by a complex model including numerous interactions. In general, the turn angle is higher in the context of random intervals compared with real excursions and in animals experiencing host encounter compared with the ones searching without success. In this respect, the difference related to patch quality is far more pronounced in the context of excursions than within the comparison of the randomly created intervals. The turn angle prior to an excursion drops significantly over time leading almost to a bisection in the turn angle, but only in those animals with host encounter. Returning to the patch leads to a re-intensification in tortuosity irrespective of patch quality. Independent of patch quality, the difference between the interval preceding and succeeding an excursions increases. It should be noted that the slight decrease in velocity at later times as well as the increase in tortuosity in the wasps encountering an empty patch contrast the initial expectations. However, the data should probably be taken with caution at the far end of the right site, as only very view animals contribute here. Thus the last 9*2 data points comparing walking behaviour before and after an excursion belong to only two animals within the animals searching on an empty patch and seven out of the last nine observations in the group with host encounter are again added by only two animals.

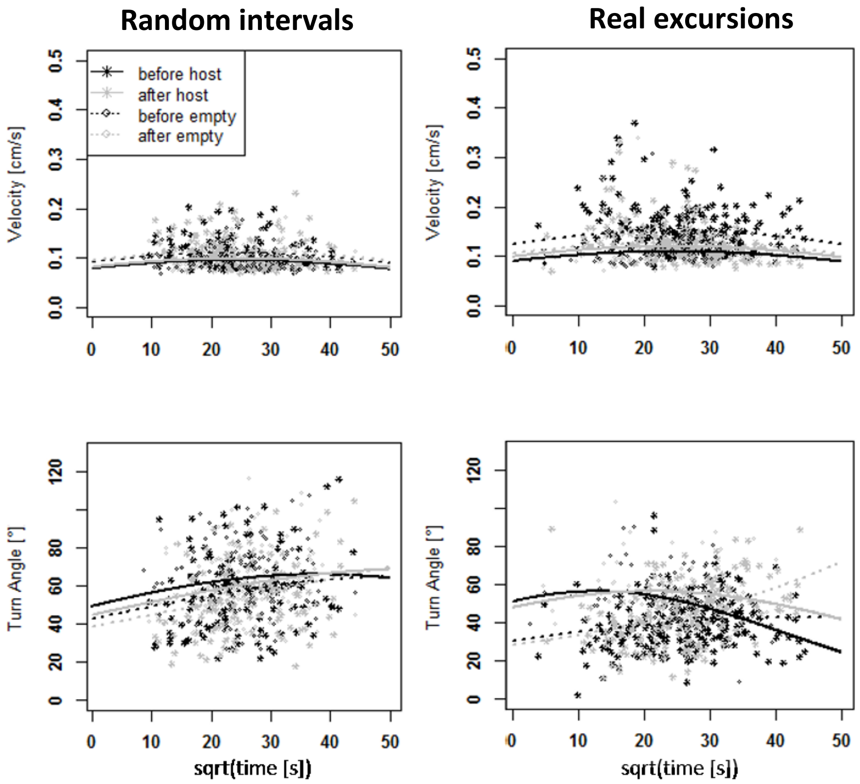


Figure 2-8: Changes in the walking behaviour in *L. heterotoma* within the 10s before (black) and the 10s after (grey) an excursion as a function of current patch time [s]. To achieve normality time on patch was square root transformed prior to analysis. Upper graphs refer to the changes in the walking velocity [cm/s], lower graphs to the changes of the turn angle [°], of the search path, measured as the change in direction per frame of the videographed search path. The left column refers to randomly chosen time intervals without an excursion, the right column to real excursions. Dashed lines refer to wasps searching on an empty patch, solid lines to wasps with host encounter. Lines refer to the line of best fit based on GLMM (see table 2-4).

2.5 Discussion

The aim of this study was to test how different events that occur while a parasitoid forages on a host patch influence its walking behaviour in the process of searching for hosts. These behavioural responses can be seen as a correlate of the forager's motivation to search a patch and, thus, the walking behaviour should be a key element in elucidating search motivation. Recently, it has been shown that an animal basically conducting a composite random walk is able to exploit patches of different quality such that it would leave at the optimal value of marginal returns, if it adapts its movement pattern mainly in the course of prey encounters (Wajnberg et al., 2013) thereby modulating a general decrease in searching intensity over time with experience. The experimental approach employed made it possible to disentangle changes in walking behaviour due to ultimate factors (like increasing intra-prey distance) from proximate event-induced changes in walking behaviour as a proxy for mechanisms regulating the so-called “searching motivation” (Lefebvre et al., 2007; Louapre et al., 2011; Pierre, 2011).

2.5.1 Response of *V. canescens*

A prerequisite for analysing the walking behaviour was to test the expectations with respect to the effect of different forms of experience during foraging on the tendency to stay on a patch. Based on host distribution under natural conditions, a decremental effect of ovipositions was expected for *V. canescens* (Driessen & Bernstein, 1999; Driessen et al., 1995; Iwasa et al., 1981) and reported by the data. Additionally a strong trend indicating a decremental effect of excursions on the tendency to stay was also found (see Figure 2-1). Accordingly, a decrease in searching intensity in the case of ovipositions and of excursions was expected as well. However no unanimous reflexion of the effects on the leaving tendency in locomotory behaviour was found. Although velocity increases over time (see Figures 2-3 and 2-4) and searching intensity is generally lower in animals searching on an empty patch (see Figure 2-4), indicating a sensitivity of searching intensity to patch quality and experience, the distinct behavioural responses towards different experiences during the search are only met in velocity but not in turn angle. Interestingly, the effect of host encounter on velocity is time-dependent and the walking velocity after a host encounter increases with time spent on patch. One possible explanation would be that with increasing time on the patch information about patch quality becomes more reliable and that this could explain why the wasps respond more strongly with increasing patch residence time. However, if this explanation would hold true, one should expect that the effect of host encounters on the tendency to remain on the patch would be a function of patch residence time as well, with stronger increments over time. Yet, this was not the case.

Excursions were preceded by a decreasing searching intensity expressed in both variables (turn angle and velocity) compared to the corresponding random intervals. This indicates that excursions are not the result of an incidental blundering off the patch, but are actually initiated even before leaving the patch. Returning to the patch, however, does not trigger any behavioural adaptations. In contrast to initial expectations, the turn angle increases over time. Generally, tortuosity is assumed to correlate positively with searching motivation (Hills, 2006; Wajnberg et al., 2013) and, accordingly, the result is counterintuitive. This relationship may either be due to more frequent encounters of the patch border over time, resulting in the reported strong turn inwards (Waage, 1978; 1979) or be an artefact caused by the increase in velocity: The turn angle is measured per frame by the observational program and this may lead to an overestimation of the turn angle especially if the animal initiates more steps than just one per frame.

2.5.2 Response of *L. heterotoma*

It was expected that ovipositions would influence the tendency to stay on the patch in *L. heterotoma* positively and excursions would influence it negatively (Haccou et al., 1991; Iwasa et al., 1981; Varaldi et al., 2005). However, no significant effect of ovipositions could be found, although the effect does appear to head in the expected direction and the estimated exp(β) matches the magnitude found in other studies (Haccou et al., 1991; Hemerik et al., 1993). However, other studies also reveal ambiguous results in terms ofthe effect of ovipositions in that species (Haccou et al., 1991; Hemerik et al., 1993) but refer to (Rosenheim, 1996). It should be taken into account that females of *L. heterotoma* have only had a single oviposition experience within our experiment. This may firstly be a relatively unnatural setting as *D. melanogaster* larvae usually occur highly aggregated and secondly leads to a low encounter rate. It has been shown previously that the encounter rate may affect the leaving tendency even more than the absolute number of encounters (Haccou et al., 1991) and studies on other parasitoids suggest that they may be well able to switch from a decremental to an incremental mechanism if the outcome does not fit the expectations (Burger et al., 2006). Finally, it has to be taken into account that the effect of prey encounters should be multiplicative (Wajnberg et al., 2013) and maybe a single event is simply too little to induce a significant impact on the leaving tendency.

In contrast to ovipositions, each excursion strongly increased the leaving tendency in *L. heterotoma*. Accordingly, if walking behaviour reflects searching motivation, only minor locomotory responses to ovipositions and more pronounced ones to excursions should be found. In fact, ovipositions did not lead to any intensification in searching intensity. In contrast, an oviposition event led to a significant decrease in tortuosity. This was not found within the random intervals and could therefore not be due to a general time-dependent decrease in turn angle. Even though it appears to contradict expectations at first glance, this pattern may be adaptive, as it prevents re-attacking the recently parasitized larva and subsequently losing time due to handling time needed to discriminate host quality. In line with the results of the tendency to stay, the walking behaviour of *L. heterotoma* is more sensitive to excursions than to ovipositions. As in *V. canescens*, searching intensity is significantly lower in animals searching on an empty patch compared to the ones with host contact (see Figure 2-8) and preceding a real excursion than within a corresponding random interval. Re-encountering the patch leads to a re-intensification in searching intensity and this effect increases over time and with waning intensity prior to the excursion. Finally, it should be pointed out that the slope of tortuosity differs significantly between animals perceiving different patch qualities. Animals with host encounters have a very tortuous walking path in the beginning that becomes straighter relatively fast, after just approx. 400 s, while in animals faced with an empty patch no such effect is seen. In those animals, as in *V. canescens*, a slight increase in tortuosity is found in contrast to the initial expectation.

To summarise, the data collect report neither the expected general switch of the walking pattern from a slow and tortuous to a faster and straighter movement nor the modifications in response to the encounter of a prey item (Wajnberg et al., 2013) and walking behaviour in the vicinity to the patch's edge seems to be more sensitive to changes.

Wajnberg et al. (2013) substantiate the locomotory effect of ovipositions based on experiments on *Trichogrammatidae* (Gardner & van Lenteren, 1986). However this oviposition-induced arrestment response was only found if the oviposition experience was not combined with host-related contact kairomones. If kairomones were present, they induced a much stronger arrestment response in the parasitoids compared to the oviposition experience and, additionally, ovipositions in combination with kairomones did not cause any additional intensification of searching intensity (Gardner & van Lenteren, 1986). The lack of host-related cues, which should define the borders of a patch (Godfray, 1994), may also explain the arrestment response following an oviposition in this family of parasitoids in other experiments (Wajnberg et al., 2003).

In the experimental set up, *L. heterotoma* in particular should have strong expectations regarding host availability due to the presence of host kairomone and their pre-experimental experiences. However, the actual number of rewards does not correspond to this expectation. It is known in detail that reliable expectations in respect to the outcome of a given situation may lead to strong neurological activity in vertebrates. Within those experiments, the actual reward fails to elicit an additional neurological response (Schultz et al., 1997; Wise, 2004), which seems to be quite similar to the pattern revealed here. Furthermore, strong mismatches of reward expectation and success lead to an inhibition of the neurological parts responsible for reward (Schultz et al., 1997; Wise, 2004), causing a negative reaction. A similar pattern has been found in *L. heterotoma* (Papaj et al., 1994). If a host encounter on a patch associated with the presence of the host fails to appear, they strongly avoid similar patches subsequently. This underlines the role of negative learning and the relation of expectation and experience in this species. Taken together, it may well be that the expected intensifying response in *L. heterotoma* fails to appear because during search time negative information accumulates lowering the reward value of a host encounter in the framework of overall patch quality. Against the background of reward and reward prediction, the strong effect of excursions on walking behaviour in *L. heterotoma* becomes adaptive too. As the patch borders define the reward predicting area, they should elicit the strongest responses if expectations rather than rewards themselves modulate behaviour.

In contrast to *L. heterotoma, V. canescens* did display a clear behavioural response to ovipositions, but no clear changes after excursions. This also makes sense against the framework of reward and reward prediction, as they did not have any pre-experimental session to allow them to construct an estimate of patch profitability. In contrast to *L. heterotoma,* where females are initially quite reluctant to respond to an offered patch but quickly respond after some positive experience (Perez-Maluf et al., 2008; van Lenteren, 1976), thelytokous *V. canescens* usually respond quickly to semolina contaminated with hosts. This makes sense as within anthropogenic habitats not much variation is to be expected between host-substrate associations. However, host density differs notably between generations depending on human impact (Skovgard et al., 1999). While host density may rise to enormous numbers in unmanaged environments, pesticides may decrease the number of hosts tremendously. Accordingly, although the association of host-contaminated grain products with hosts should be very strong leading to short latencies, the assessment of patch quality should undergo some learning process. As long as no reliable patch assessment took place, the reward rather than the reward predictor should elicit a response, as it was found consistently in the analyses of the leaving tendency as well as of the locomotory response.

To conclude, the general context of this work is the link between ultimate predictions in respect to fitness consequences and proximate mechanisms. The results have shown that patch time allocation in two insect parasitoid species cannot easily be explained by the simple result of kind of composite random walk (Benhamou, 2007; Fronhofer et al., 2013; Wajnberg et al., 2013) even though some aspects of such random walking models are retrieved like the general decrease in searching intensity over time. Furthermore, different species obviously respond with different locomotory reactions and, moreover, pre-patch experiences most likely play a substantial role in the behaviour expressed on a patch. This supports the role of some kind of learning process in the modulation of searching process rather than a simple universal movement rule.

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Chapter 3

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Effects of octopamine-feeding on responsiveness to the patch in the parasitoid Leptopilina heterotoma

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3.1 Abstract

The neuromodulator octopamine (OA) is crucial for numerous functions ranging from responsiveness to different cues and learning to foraging behaviour. While OA enhances responsiveness and the likelihood of learning in appetitive learning paradigms especially, it is found to shift foraging behaviour towards a more explorative behaviour characterized by shorter searching bouts and a higher frequency of patch changes in foraging experiments. Those findings are contradictory based on patch leaving models, which assume a decreasing responsiveness as the key mechanism for patch leaving, as then longer patch residence times caused by higher responsiveness should be expected.

This study tested the effect of oral OA treatment on searching behaviour in the parasitoid *Leptopilina heterotoma* focusing on the time needed to enter a patch containing hosts, patch residence time, and searching efficiency. We found that OA-treated animals needed substantially longer to enter the patch while no effects on patch residence time were found. However OA treatment led to a slightly higher efficiency in patch exploitation.

3.2 Introduction

The neuromodulator octopamine (OA) is widely accepted to be the key substance in the modulation of responsiveness towards appetitive stimuli and in reward learning in bees (Barron et al., 2002; Hammer & Menzel, 1998; Mercer & Menzel, 1982; Scheiner et al., 2002; Schulz et al., 2002), d*rosophilids* (Schwaerzel et al., 2003; Scheiner et al., 2014) and crickets (Mizunami & Matsumoto, 2010; Nakatani et al., 2009; Unoki et al., 2005). In this respect, intrinsic OA concentrations generally seem to correlate positively with responsiveness (Barron et al., 2002; Pankiw & Page, 2003; Scheiner et al., 2014) and learning performance (Behrends & Scheiner, 2012). In addition, recent research into bees indicates a crucial role of OA in foraging behaviour (Liang et al., 2012). OA was found to induce scouting behaviour, which is expressed, amongst other things, in shorter searching bouts on a given patch and more frequent patch transitions (Liang et al., 2012). Furthermore, OA was shown to decrease the intensity of searching behaviour in terms of movement pattern and patch residence time in *Manduca sexta* (Nathanson et al., 1993).

Those findings in the two complexes of learning and responsiveness on the one hand and foraging behaviour on the other hand are conflicting based on patch time allocation models. These often propose a mechanism combining an initial level of responsiveness to patch associated cues and its subsequent waning finally leading to patch abandonment (Driessen et al., 1995; Iwasa et al., 1981; Thiel & Hoffmeister, 2004; Waage, 1979; Wajnberg, 2006). The level of initial responsiveness in combination with the reward induced increments determine the patch residence time (Driessen et al., 1995; Iwasa et al., 1981; Thiel & Hoffmeister, 2004; Waage, 1979; Wajnberg, 2006) and the higher the responsiveness, the longer it should take the animals to abandon a patch. Accordingly, it could be argued that if OA is crucial in setting the levels of responsiveness then intrinsic concentrations of OA should positively correlate with patch residence time. However, studies on foraging behaviour indicate that the opposite effect should be expected (Liang et al., 2012; Nathanson et al., 1993). This raises the question of how OA modulates foraging behaviour in species other than bees.

Although a plethora of studies investigated how parasitoids allocate their patch residence time according to physiological and environmental circumstances (e.g., Driessen & Bernstein, 1999; Driessen et al., 1995; Outreman et al., 2005; Thiel & Hoffmeister, 2004; van Alphen et al., 2003; Visser et al., 1992; Wajnberg, 2006), optimal adaptation of patch residence times is only part of a successful searching strategy and animals need to develop ways to locate suitable patches efficiently within a complex environment. However, microhabitat location was focused on far less by behavioural ecologists (but see, e.g., Fleury et al., 2009; Kaiser et al., 2009; Kaiser et al., 2003; Papaj et al., 1994; Papaj & Vet, 1990; Perez-Maluf et al., 2008; Vet & Papaj, 1992). In most host-parasitoid systems, the host elicited cues are relatively non-volatile and parasitoids depend on the use of cues emitted by the host's microhabitat to orientate themselves towards a suitable patch (Vet et al., 1991). The response towards host habitat cues is not predominantly innate, but needs to be learned in a process largely mirroring classical conditioning (Fleury et al., 2009; Kaiser et al., 2009; Kaiser & Dejong, 1995; Kaiser et al., 2003; Papaj et al., 1994; Papaj & Vet, 1990; Rafalimanana et al., 2002; Vet & Papaj, 1992). A successful oviposition (the unconditioned stimulus) combined with an odour (the conditioned stimulus) leads to memorising the odour. In future, the animal will react to the odour alone. Accordingly, the intensity of this association appears to depend on the number of associative trials (Kaiser et al., 2003) and the memory can be erased to some extent if ovipositions fail to occur (Kaiser et al., 2003). One of the consequences of this learning process is an experience-dependent increase in the speed of location of suitable microhabitats (Papaj et al., 1994; Papaj & Vet, 1990; Vet & Papaj, 1992). Based on the role of OA in appetitive classical conditioning (Hammer & Menzel, 1998; Mizunami & Matsumoto, 2010; Mizunami et al., 2009; Schwaerzel et al., 2003; Unoki et al., 2005; Unoki et al., 2006) and responsiveness (Barron et al., 2002; Pankiw & Page, 2003; Scheiner et al., 2014), it is hypothesized that the time needed to enter the patch should be sensitive to OA treatment and that enhancing OA titres should enhance the speed of patch encounter. Furthermore, if OA affects only the responsiveness to patch associated odours, this effect should be especially pronounced under the direct influence of OA, but, if OA facilitates learning, the effect should be at least as pronounced on subsequent patch visits, even in the absence of further OA treatment.

Lastly, exploitation efficiency on a patch is sensitive to experience (Hemerik et al., 1993; Rafalimanana et al., 2002; Raine & Chittka, 2007). E.g., bumblebees have been shown to become more efficient in the collection of pollen with experience (Raine & Chittka, 2007). Similarly, the parasitoid *Leptopilina heterotoma* was shown to adapt its characteristic host location behaviour to the structure of the patch, leading to an increased host encounter rate (Hemerik et al., 1993) and to enhance host location efficiency in response to stress (Rafalimanana et al., 2002). Therefore, efficiency in host exploitation might be a suitable focus variable to test the effects of OA on learning performance in parasitoids.

Here theexperiment investigates the effect of oral OA application on three aspects of foraging behaviour in the parasitic wasp *Leptopilina heterotoma* (Hymenoptera: Figitidae) (THOMSON): Patch residence time, the time needed to encounter the patch (latency) and the rate of exploitation (measured as the percentage of hosts parasitized over time). To disentangle the effects of OA on responsiveness from effects on memory acquisition, all animals were given two similar patches to exploit either in quick succession or on two successive days. It was assumed (1) that the direct application of OA prior to a patch visit should lead to longer patch residence times and faster patch encounter in response to an increased responsiveness to the patch and patch-related cues and (2) that formerly OA-treated animals should perform better on subsequent patches, if it facilitates memory acquisition. This should be expressed in faster patch location and higher exploitation efficiency. However, (3) patches should be more readily left due to a mismatch of expectations and findings regarding the patch quality, as it has been shown that perceived subjective resource value correlates positively with intrinsic OA concentrations (Barron et al., 2007). Accordingly, the expected value of a patch, as estimated during a first patch visit, should be higher than what the animal actually experiences on that patch.

3.3 Materials and methods

3.3.1 Study species

The study used wasps of the solitary endoparasitoid *Leptopilina heterotoma* reared under lab conditions at 20°C and a 16:8 (L:D) photoperiod on its natural host *Drosophila melanogaster*. In this species, chemoreception is the main mechanism used in host searching, with most chemoreceptors being located at the ovipositor (Dicke et al., 1984; Dicke et al., 1985). In other insect species, it was shown that chemoreception is modulated via the octopaminergic system (Farooqui, 2007; Roeder, 1999; 2005) and it is very likely that this is conserved throughout the insect class (Bleeker et al., 2006; Farooqui, 2007), making *L. heterotoma* a promising target species for testing effects caused by OA on responsiveness in parasitoids.

3.3.2 General protocol

Rearing tubes were emptied daily to ensure wasps of the same age within the experiments. Wasps used in the experiments were separated within 24 hrs of hatching and placed in small glass vials containing two males of the same species to ensure mating. The glass vials contained a layer of agar to ensure a constant humidity within the vial and additionally to provide a water source for the insects but no additional food source to ensure feeding prior to the experiment. This treatment did not cause any increased mortality and *L. heterotoma* is more than able to survive food deprivation for more than a week (Vuarin et al., 2012). The wasps remained in the vial for another two days until they were offered the first of a total of three patches to be exploited. All patches consisted of a yeast patch measuring 2 cm in diameter, made of fresh baker's yeast (*Saccharomyces cerevisiae*) dissolved in water, placed in the centre of a Petri dish lined with agar. One hour prior to the experiments, six 2nd instar *D. melanogaster* larvae were placed randomly on the patch, which should lead to comparable kairomone concentrations on all patches. The first patch (pre-experimental patch) served as the learning patch. Additional wasps which did not oviposit within this pre-experimental treatment were discarded from the following experiment. After the training session, wasps were stored in Eppendorff cups covered with Parafilm.

3.3.3. Drug treatment

To minimize the stress for the animals, drug treatment was administered orally. Feeding of OA was shown to affect behaviour significantly (Barron et al., 2007; Cnaani et al., 2003; Liang et al., 2012; Schulz & Robinson, 2001), to produce similar results to injection experiments using a 10-fold higher concentration (Scheiner et al., 2002) and to induce significant changes in behaviour within minutes (Pankiw & Page, 2003). Sugar solution was concentrated by a rate of 1:5 and the same sugar solution was used to dilute the octopamine with a concentration of 1.2 mM. This concentration was based on the results of (Scheiner et al., 2002) for bees, corrected for the body size of our organism and the average drinking volume after the given time of deprivation. Octopamine hydrochloride was obtained from Sigma Aldrich (Germany). Feeding took place immediately before each patch visit. A little droplet of either solution was placed in the Eppendorf-cup and wasps were allowed to drink from this droplet for 15 s. This procedure allowed at least a rough comparability of the amount of chemicals incorporated. Furthermore prohibition of drinking after 15 s ensured that wasps would drink again prior to the second patch visit, which they refused especially in the short travel treatment within pre-experiments, if they were allowed to drink at libitum.

After drinking, the Eppendorff cup was placed carefully beside the patch and the wasps were allowed to enter the experimental patch freely.

3.3.4 Experimental approach

Including the pre-experimental patch, three patches were visited, each of the same quality. Prior to each patch wasps were allowed to drink a given solution for 15 s. This was always sugar solution (SU) preceding the pre-experimental patch and either SU or octopamine (OA) solution prior to the first and/or second experimental patch. Considering all the possible feeding combinations, the final result was four different feeding combinations for the second experimental patch, but only two for the first (see Table 3-1). Furthermore, waiting times in between the patch visits differed. Whereas the waiting time was always 24 hrs between the pre-experimental and the first experimental patch, waiting times differed between the first and second experimental patch and wasps had to wait either 5 min or 24 hrs (see Table 3-1).

Table 3-1: Replicate numbers and possible feeding and interval combinations. Data from the first experimental patch could only contribute to the SUSU and SUOA treatment within the long travel treatment as conditions from the pre-experimental patch to the first experimental patch were constant for all wasps.

PATCH	INTERVAL (time since last patch visit)	Possible combinations of feeding treatments SU=sugar, OA=Octopamine in brackets= N			
1	24 hrs	SUSU (43)	-	SUOA (45)	-
2	5 min	SUSU (11)	OAOA (12)	SUOA (9)	OASU (10)
2	24 hrs	SUSU (11)	OAOA (11)	SUOA (12)	OASU (11)

3.3.5 Behavioural scoring

Behaviour was scored using The Observer 2.0 (Noldus Information Technology, Wageningen, Netherlands). The test scored the time it took the wasps to enter the patch (LATENCY), whether the animal was on or off the patch as well as probing into a host, searching related behaviour (walking and pricking on patch) and not searching related behaviour (being off-patch, resting and preening). Host attacks were scored as ovipositions when probing lasted longer than 16 s (Haccou et al., 1991). However, dissecting the larvae revealed even shorter probing attempts as successful: the shortest time needed for successful oviposition was 14.8 s.

For the statistical analysis, excursions lasting longer than 30s were defined as the point of final patch leaving. However, this was not always clearly distinguishable within the running experiment. To ensure that no trials would be terminated too soon, observations were terminated after 1 min off the patch. Wasps were placed in an Eppendorff cup again and the procedure was repeated either after 5 min or roughly 24 hrs, depending on the assigned travel time.

3.3.6 Data Analysis

Data were analysed using Cox proportional hazard analyses using the *coxme* package for Mixed Effects Cox Models (Therneau, 2012) in R 2.15.2 (R-Core-Team, 2014). As all wasps were observed twice (with one exception refusing to search on the 2nd PATCH) WASPID was specified as the random term. The best model was chosen based on the AIC and stepwise backwards elimination of terms worsening the explanatory power of the model.

The first test was on the impact of food given before the previous patch visit (FOOD1), food given before the current patch visit (FOOD2), and time since last patch visit (INTERVAL) up to their three-way interaction on the time needed to enter the patch (LATENCY). Additionally, the number of patch visits (PATCH) was taken into account.

The second test ran a similar model to investigate the impact of the abovementioned explanatory variables on the patch residence time (PRT). In addition to the mentioned fixed terms (FOOD1, FOOD2, INTERVAL and PATCH), ovipositions (OVI) were added as a time-dependent variable. The full model was based on all possible three-way interactions of OVI, FOOD1, FOOD2 and INTERVAL plus PATCH as a single term.

Finally, an analysis was performed of the rate of patch exploitation over PRT by means of generalized linear mixed models (GLMM) using the library *lme4* (Douglas Bates et al., 2014). A sigmoidal model was fitted to the percentage of hosts parasitized over PRT starting again with all possible three-way interactions of OVI, FOOD1, FOOD2 and INTERVAL and PATCH as additional factors.

3.4 Results

3.4.1 Effects of OA treatment on latency

Table 3-2 shows the effects of all variables remaining in the final model based on the AIC criterion. The overall fit of the model is highly significant ($\chi 2$=27.36, *df*=5, *p*=4.85e-05). Feeding treatment best explained latency. Recent OA feeding (FOOD2) in particular led to longer latencies (see Figure 3-1, Table 3-2). Although not as expressed in the animals experiencing short travel times, prior OA and subsequent SU feeding

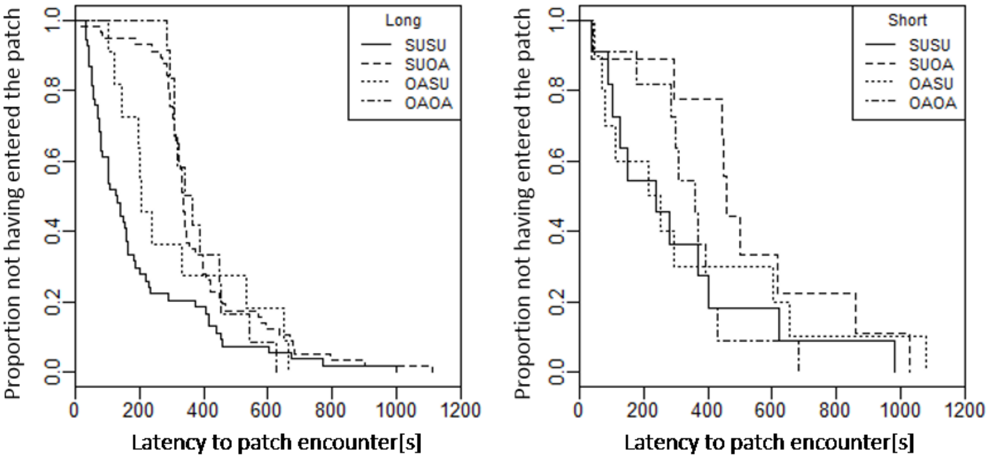

Figure 3-1: Kaplan-Meier curves describing the proportion of *Leptopilina heterotoma* females not having entered the patch after a given time [LATENCY (s)] as a function of INTERVAL (short and long waiting time between patch visits) and feeding treatment with sugar and octopamine before the first and second patch visit. For levels of significance see text.

Table 3-2: Estimated regression coefficients (β), standard errors (se) and hazard ratios (exp(β)) for those covariates and interactions that substantially increased the explanatory power of the model based on AIC on the tendency to enter the patch (LATENCY)

	β	**se**	**exp(β)**	**z**	***p*-value**
FOOD1(OA)	-0.58	0.33	0.56	-1.75	0.079
FOOD2(OA)	-1.14	0.23	0.31	-5.05	4.3e-07
FOOD1+2(OA)	-0.88	0.43	0.41	1.95	0.051
INTERVAL (SHORT)	-0.37	0.22	0.69	-1.65	0.1
All of them were computed with all the other terms present in the model. SUSU-treated animals experiencing long inter-patch intervals were assumed to be the reference level (i.e., baseline hazard with a coefficient set to zero). Overall fit of the model: *χ2*=27.36, *df*=5, *p*= 4.8489e-05.					

increased LATENCY in general as well. Intra-patch INTERVAL should, although not significant (see Table 3-2), remain in the model based on the AIC criterion. This effect is independent of feeding treatment and short INTERVALS slightly increase the time needed to enter the patch. PATCH number did not affect latency at all (*χ2*=0.05, *df*=1, *p*=0.83) nor did any other interaction.

3.4.2 Effects of OA treatment on PRT and on-patch behaviour

Patch residence time was best explained by ovipositions alone (OVI: β=-0.18, se(β)=0.07, exp(β)=0.83, z=-2.63, χ^2= 6.63, df=1, p=0.01), meaning that each oviposition decreased the tendency to leave by 16.8%. The effect of ovipositions was not affected by feeding treatment, as all interactions turned out to be insignificant (OVI*FOOD1: χ^2= 1.4, df=1, p=0.24; OVI*FOOD2: χ^2=1.09, df=1, p=0.3; OVI*FOOD1*FOOD2: χ^2=1.38, df=1, p=0.24). Neither INTERVAL (χ^2= 0.05, df=1, p= 0.81) nor PATCH (χ^2= 1.85, df=1, p= 0.17) or food in any combination (FOOD1: χ^2=0.2, df=1, p=0.65; FOOD2: χ^2=0, df=1, p=0.97; FOOD1*FOOD2: χ^2= 0.11, df=1, p=0.74) nor any other interaction term increased the explanatory model significantly and the median patch residence time is irrespective of feeding treatment or INTERVAL around 1060 ± 35s (SE) on patch (see Figure 3-2 left).

However, behaviour on the patch differed significantly depending on FOOD. Prior OA feeding (FOOD1, see Figure 3-2 right, green lines) significantly increased the rate until which the patch was exploited (FOOD1: χ^2=7.07, df=1, p=0.01), leading to a significantly steeper slope of the proportion of hosts successfully attacked (FOOD1*logPRT: χ^2=7.81, df=1, p=0.005, see Figure 3-2 right) compared to sugar treated wasps (black). Additionally, animals experiencing quick patch succession (dashed lines / filled circles) are significantly more efficient in parasitizing the offered hosts compared to the ones experiencing long INTERVALS (solid lines / open circles) between two patch encounters (INTERVAL: χ^2=11.50, df=1, p=0.0006, see Figure 2 right). This effect was irrespective of feeding treatment as all interactions of INTERVAL and FOOD were insignificant as well as all other variables tested except for logPRT alone, which was, logically, highly significant (χ^2=117.87, df=1, p< 2.2e-16).

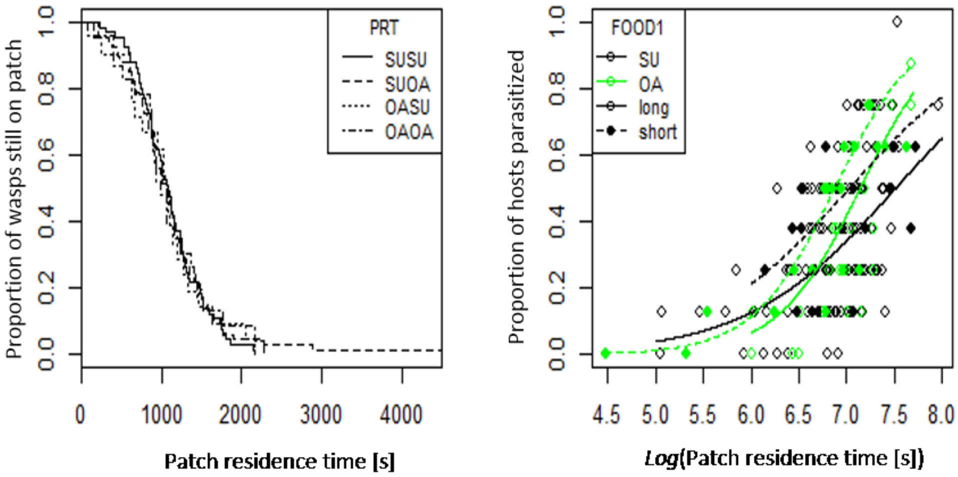

Figure 3-2: Left: Kaplan-Meier curves describing the proportion of Leptopilina heterotoma females still on the patch as a function of time (patch residence time [s]), and feeding treatment (see legend). Right: Effect of feeding treatment prior to the previous patch visit (FOOD1) and waiting time in between the two patch visits (INTERVAL) on the proportion of hosts successfully attacked over time *log*(patch residence time [s]). SU = black, OA = green, long intervals = solid lines / open circles, short intervals = dashed lines / filled circles. Lines of best fit retrieved from GLMM analysis with binomial distribution. For levels of significance see text.

3.5 Discussion

We tested the effect of OA feeding on foraging behaviour in *L. heterotoma*, with a focus on patch residence time (PRT), time needed to encounter the patch (latency) and searching efficiency. It was assumed that (1) recent OA treatment enhances the responsiveness to the patch (van Alphen et al., 2003) or its edge (Waage, 1978; 1979) leading to faster patch encounter and longer PRT. Furthermore, the animals were tested twice to unravel OA-related effects on patch quality learning from momentous shifts in responsiveness unrelated to patch quality assessment. It was assumed that (2a) learning about the patch characteristics should make the animals more efficient during their search, (2b) if OA promotes associative learning, this effect should be especially pronounced in previously OA-treated animals leading to a higher searching efficiency and shorter latencies but (3) to shorter patch residence times on subsequently encountered patches of the same quality in the absence of OA-treatment due to its role in reward value assessment. This hypothesis was based on the assumption that increasing intrinsic OA concentrations should increase the subjective value (Barron et al., 2007) of a patch. A second patch is then encountered with high expectations regarding its quality which is not congruent with the findings.

Our results did not unanimously meet our expectations. For the latency to enter a patch the opposite of the expected pattern was found and OA treatment increased the time needed to initiate searching activity on the patch highly up to almost twice the time needed in untreated wasps (see Figure 3-1, Table 3-2). Furthermore, this effect is mostly promoted by recent OA treatment and food received prior to the previous patch visit only has minor effects, albeit heading in the same direction (see Figure 3-1, Table 3-2). This clearly contradicts the hypothesis as it was assumed especially recently OA-treated animals would quickly perceive and encounter the patch based on the reported effects of OA on lowering the response thresholds (Barron et al., 2007; Barron et al., 2002; Erber & Kloppenburg, 1995; Scheiner et al., 2002; Scheiner et al., 2014). In addition and no less puzzling, animals experiencing short intra-patch intervals responded with prolonged latencies as well. Once again, the opposite was expected, as these animals have had a very recent, probably rewarding experience with the experimental setup and it has been shown that especially recent experiences are used to enhance subsequent performance (Raine & Chittka, 2007). It might be argued that this prolonged response time could be explained by physiological constraints caused by ongoing searching activity, like, e.g., muscle fatigue, egg depletion or detrimental effects of the OA treatment, plus it might have been caused by a partial habituation to the patch-related odours. However, all this seems to be very unlikely, as, firstly, once on a patch, no treatment specific differences were found in patch residence time and, what is more, both OA-treated and animals experiencing short travel times were more efficient in patch exploitation on subsequent patches, which contradicts any physiological constraints. Lastly, no OA-specific detrimental effects on motor abilities have been found to date (Jones et al., 2011; Liang et al., 2012; Menzel, 1999), *L. heterotoma* has an initial egg load of roughly 150 eggs (Le Lann et al., 2014), which is far more than they could have laid here, and, finally, partial habituation can probably be excluded from explaining effects of short intra-patch intervals on searching behaviour (Thiel, 2011).

Patch residence time was not affected by any other variable but ovipositions (see Figure 3-3 left). This finding contradicts the team's first and its third hypothesis and, furthermore, earlier studies testing the effect of different intra-patch intervals on patch residence time (Tentelier et al., 2006; Thiel, 2011; Thiel et al., 2006; Thiel & Hoffmeister, 2004). Yet, even in these experiments, the expected effects of travel time either became just visible over a number of patches (Thiel et al., 2006; Thiel & Hoffmeister, 2004), were masked by high intra-individual variance (Thiel, 2011) or only showed a weak positive correlation of travel time and patch residence time (Tentelier et al., 2006). It needs to be considered that experiencing a short intra-patch interval in our experiment was not a very reliable piece of information for the wasps, as the preceding interval, ranging from the pre-experimental to the first experimental patch, was considerably longer, so overall the information sampled in respect to habitat quality was rather variable and the experience of a single short intra-patch interval is probably not sufficient to serve as a reliable indicator for an increasing patch density. To go a step further, in the light of high variability in patch encounter rates, a fast adaptation in patch residence time would be likely maladaptive as the value of information with regard to future expectations is only relatively low (Stephens, 1993).

OA treatment did not cause any significant effects with regard to patch residence times. As mentioned, longer patch residence times were expected as a result of an increased responsiveness induced by OA treatment (hypothesis 1), which has been shown within a number of circumstances in bees and drosophilids, though mostly in the context of nutritional rewards (Barron et al., 2007; Barron et al., 2002; Erber & Kloppenburg, 1995; Scheiner et al., 2002; Scheiner et al., 2014). Moreover, a drop in patch residence time was expected if a first patch was visited under the influence of OA but the latter not (hypothesis 3). This hypothesis was related to findings in which honey bees rank the profitability of a patch higher under the influence of OA (Barron et al., 2007). As parasitoids have been shown to react with shorter patch residence times if preceding patch quality was better (Tentelier & Fauvergue, 2007), it was assumed that OA treatment would mimic a similar pattern.

It is possible that the reasons that none of the hypotheses could be confirmed are similar to the not retrieved effect of travel times on patch residence time. Likely irrespective of responsiveness and subjective value of the patch, the intra-individual variance was too high and the amount of information too low to reveal measurable adaptations, even though individual differences were considered statistically by adding *waspID* as the random term. Furthermore, it might be the case that patch time allocation in response to host kairomones and rewards in forms of oviposition are not modifiable by OA treatment, perhaps because the response is too hard-wired due the tight link of host finding and fitness (Godfray, 1994) or simply that another neuronal pathway is involved in the sensory integration of hosts and host-related cues and actually it has been criticised that most studies on the role of OA in reward learning and responsiveness were conducted on sugar reward, making it difficult to generalise (Perry & Barron, 2013) and actually the role of OA in other reward schedules could not be univocally retrieved (Sitaraman et al., 2010) but see (Erber & Kloppenburg, 1995; Unoki et al., 2006). However, OA treatment has had a substantial effect on foraging behaviour in *L. heterotoma*, as previous OA treatment made them more efficient during their search on subsequent patches (see Figure 3-2 right). An increase in efficiency is a clear sign of learning with experience (Chittka & Muller, 2009; Raine & Chittka, 2007), which has been found in *L. heterotoma* before (Hemerik et al., 1993) and the fact that this finding is not simply an artefact is even underlined by the finding that animals experiencing quick patch succession performed better as well. A similar pattern in respect to time interval has been found in bumblebees (Raine & Chittka, 2007). They became clearly more efficient in a foraging task during the day, although overnight some of the increase in performance vanished indicating imperfect memory retention, similar to the pattern found here. Improved learning via OA injections has been found, e.g., in bees (Behrends & Scheiner, 2012; Menzel et al., 1999). Though these findings support the expectations regarding the effects of experience and OA treatment on efficiency (hypotheses 2a and b) it must not be neglected that they are inconsistent with the other findings, especially with respect to the latency to encounter the patch. So far, it is only possible to speculate on the reasons. It should be considered that the treatment given added OA artificially to the natural level, thereby likely shifting the OA level above an optimal threshold. It has been suggested that although OA is essential for memory acquisition, an artificially increased level may have detrimental effects, leading to a more aroused behaviour (Menzel et al., 1999) and higher distractibility. Additionally, OA is the central modulator of the invertebrates' stress response (Adamo & Baker, 2011; Adamo et al., 2013; Harris & Woodring, 1992; Roeder, 1999; 2005). In humans, stress leads to a higher unselective alertness (Starcke & Brand, 2012), similar to increased arousal. Moderate levels of stress may actually increase performance, probably due to increased alertness, on the one hand, whereas on the other they may cause impairments to the learning of cues which are relevant to making the optimal decision. With respect to the set-up of the experiment, it would probably be naïve to assume that the handling of the specimens does not serve as an additional stressor for the animals. In the light of OA modulating the stress level, it is likely that OA-treated animals need longer to recover from the stress caused by the handling leading to prolonged latencies, while the same increase in stress level leads to an elevated efficiency in patch exploitation. An increased searching efficiency (Rafalimanana et al., 2002) and a more intense searching pattern (Delpuech et al., 2005) as a result of sublethal physiological stress have been reported for *L. heterotoma* before, underlining that searching behaviour on the patch is sensitive to experience in this species.

Initially, this paper mentioned results on OA treatment showing a more explorative behaviour (Liang et al., 2012) and less intense searching pattern (Nathanson et al., 1993). The results collected do not retrieve any related effects in the system used here and it remains open to speculation, if the effects found in that studies were specific to the given system used.

To conclude, this study served as an approach to link the field of optimal foraging research with the knowledge of insect physiology, especially the octopaminergic system. The necessity to build this bridge has recently been underlined by numerous authors (Hoedjes et al., 2010; McNamara & Houston, 2009; Pierre, 2011). The results collected show that the behaviour of *L. heterotoma* is sensitive to aminergic treatment. Thereby OA seems to enhance learning capabilities once the animals entered the patch but leads to a suboptimal performance in the ability to locate a patch with hosts quickly. We hypothesize that OA treatment mimics stress, as stress can induce suboptimal learning on the one hand but may increase efficiency in a known set-up on the other. The results collected cannot be explained by pure shifts in response thresholds. This may be explained by the reward chosen here. While most studies use nutritional rewards to measure responsiveness, this studied used hosts of the parasitoids, encountering which should be highly rewarding. As the results reveal a complex pattern, parasitoid-host systems may be a useful system for filling in the criticised lack of information concerning the role of OA in the modulation of reward perception and responsiveness in other than nutritional contexts (Perry & Barron, 2013).

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Chapter 4

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Octopamine affects the dynamic response to danger in a parasitoid wasp

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4.1 Abstract

The function of neuromodulators in behaviour has been widely discussed in a number of invertebrates, but relatively little is known about their effects in insect parasitoids. This is somewhat surprising, as very clear assumptions of optimal decision-making in these species exist. This study tests the effect of orally administered octopamine and dopamine on dynamic decision-making in the parasitoid *Asobara tabida*. The parasitoids' patch-leaving decision in the present set-up needs to be coevally balanced with respect to appetitive (oviposition opportunities) and aversive stimuli (mimicked presence of a predator) on a depletable patch. As expected, the results found an increasing number of wasps leaving the patch in response to the aversive stimulus with an increasing rate of exploitation. This shape was highly age-dependent, with younger wasps showing no adaptation to the rate of exploitation. Orally administered dopamine did not generally change this pattern. In contrast, animals fed octopamine reacted with a higher chance overall of leaving the patch in response to the mimicked danger cue, which was irrespective of age and the rate of exploitation. It was concluded that this result indicates an increased stress level caused by octopamine treatment rather than stimulus-specific modulations of responsiveness.

4.2 Introduction

4.2.1 Dealing with risk

Most animals face a severe risk of predation in the wild. This holds particularly true for small, defenceless creatures like insect parasitoids (Brodeur & Rosenheim, 2000; Heimpel et al., 1997; Meyhofer & Klug, 2002; Rosenheim, 1996). Nevertheless, minimising predation risk is always associated with costs as “a hiding animal is not a foraging animal” (Nonacs, 2010) and therefore predator defence decreases lifetime foraging success, which is reflected, for example, in fewer oviposition events in the case of parasitoids. An optimal forager should only decide to react with anti-predator behaviour if the costs of this behaviour are lower than the expected benefit from not reacting (Nonacs, 2010; Stephens & Krebs, 1986). For example, leaving a good patch could be costly and costs are even higher if patches are rare (Nonacs, 2010). On the other hand, it could be the better strategy to leave a patch upon the arrival of a competitor, if it is already exploited to a broad extend (Kotler et al., 2004). How animals adjust their behaviour in order to perform optimally in a framework of managing multiple goals can be examined, for example, by studying the giving-up densities (how much prey is left on patch under different risk regimes) (Kotler et al., 2004) or similarly by behavioural titration (Abrahams & Dill, 1989). Astonishingly, even though patch time allocation has been extensively studied in parasitoids as their searching success is directly linked to fitness (Godfray, 1994) dynamic trade-offs in the face of danger have only rarely been studied in these organisms. Recently, Roitberg et al. (2010) showed that the solitary endoparasitoid *Asobara tabida* adapts its behaviour according to the rate of patch depletion when confronted with a puff of formic acid to mimic the arrival of a predator: If they had only recently arrived, wasps remained on the patch, but with an increasing rate of exploitation and therefore decreasing host finding rate, the behavioural response switched and the wasps left.

4.2.2 Age

One main concept of dynamic decision-making is that the net benefit of a given behaviour is not constant but varies with the physiological and environmental circumstances as, for example, the costs of leaving the patch decrease with the rate of exploitation (Roitberg et al., 2010). Accordingly, animals behave differently in respect to the given situation as has been shown in a variety of species and physiological as well as environmental factors. For instance, ageing leads to longer patch times in *Asobara tabida* (Thiel & Hoffmeister, 2004) as well as in other parasitoids (Thiel et al., 2006; Wajnberg, 2006) but see (Jenner et al., 2012). This is usually explained by more pessimistic expectations regarding the chances of finding another patch (Thiel & Hoffmeister, 2004; 2009). Transferred to the dynamic quality-dependent response to danger, this would mean that younger wasps should be less likely to stay in the face of danger, as they should have a longer life expectancy and therefore higher chances of finding other patches compared to older wasps or, to put it into economic terms: the costs of leaving should be lower for younger wasps than for their older conspecifics.

4.2.3 Neuronal modulation of decision making

While the ultimate causes of dynamic decision-making are thoroughly discussed (Stephens & Krebs, 1986; Thiel & Hoffmeister, 2009; Wajnberg, 2006), the proximate ways of decision-making remain elusive, especially in parasitoids. This is astonishing, as, based on the close linkage between foraging success and fitness, parasitoids should be excellent model organisms not just for ultimate but also for proximate foraging-related research questions (Hoedjes et al., 2010). Neuromodulation allows short-term adaptation to different physiological and environmental conditions without changing the “hard-wire” (Birmingham & Tauck, 2003). Basically, neuromodulators may change receptor sensitivity, neuron firing rate or shift thresholds (Birmingham & Tauck, 2003) and this may lead to changes in behaviour. In many invertebrates, the biogenic amine octopamine (OA) deals as a neuromodulator in a variety of neuronal circuits (Birmingham & Tauck, 2003; Farooqui, 2007; Roeder, 1999; Scheiner et al., 2006) and seems to play a key role in general arousal (Behrends & Scheiner, 2012; Harris & Woodring, 1992), stimulus perception (Mercer & Menzel, 1982; Scheiner et al., 2002) and reward learning (Hammer & Menzel, 1998; Matsumoto et al., 2003; Nakatani et al., 2009; Unoki et al., 2005) as well as in stress-dependent decision-making (Adamo et al., 2013; Adamo et al., 1995; Rillich et al., 2011; Stevenson & Rillich, 2012). Despite the variety of octopaminergic effects found in flies, crickets and honey bees, only one study focuses on the octopaminergic system in parasitoids: Recently, an octopamine activity pattern similar to those of honey bees was found in the brain of two parasitoids of the genus *Cortesia* (Bleeker et al., 2006).

Another biogenic amine, dopamine (DA), is classically meant to act as a kind of OA antagonist. While OA is assumed to modulate reward learning, DA is assumed to be crucial for aversive learning (Agarwal et al., 2011; Nakatani et al., 2009; Schwaerzel et al., 2003; Unoki et al., 2005). However, evidence is appearing that the relation of OA mediating the effect of “good” and DA of “bad” stimuli may be too short cut (Perry & Barron, 2013). Instead, DA may like OA (Behrends & Scheiner, 2012) be more involved in arousal and goal specific sensitivity (van Swinderen & Andretic, 2011) and interactions of both aminergic systems may be much more complex than actually thought. This complexity is supported by studies showing the role of DA even in appetitive learning paradigms (Liu et al., 2012; Marella et al., 2012), which contradicts the classical view. One problem in disentangling effects of general arousal from stimulus specific responses may be that in most studies animals do not have to balance their decision with respect to aversive and appetitive stimuli. It has been suggested that the difficulties in learning within an aversive learning experimental set-up in DA-manipulated flies may be due not to a lack of perception of the aversive stimulus per se but due to shifts in general arousal, which may result in a higher responsiveness to irrelevant stimuli and therefore in worse performance in the experiment (van Swinderen & Andretic, 2011).

The dynamic trade-off experimental set-up (Roitberg et al., 2010) where the animal has to weigh up the risk and benefit reveals the possibility of disentangling effects on appetitive and aversive stimuli as both are present in the combined set-up. Arrival on patch and subsequently host findings should definitely be a rewarding experience, whereas a puff of formic acid should be a highly aversive stimulus (Roitberg et al., 2010). If OA is specific to positive stimuli, they should stay longer even in the face of danger, as the value of the patch should be estimated to be higher. On the other hand, if OA increases sensitivity in general, increased OA may even lead to faster patch abandonment. Artificially elevating the DA level should, following the classical view of modulation of the perception of aversive stimuli, lead to premature leaving decisions.

4.3 Materials and Methods

4.3.1 General experimental set up

Basically our experimental set up equals the one described in (Roitberg et al., 2010). Females of the larval endoparasitoid *Asobara tabida* were released into a known experimental arena, which was a 4.3cm Petri dish lined with agar. In the centre of the dish was a patch consisting of 45 µl of a vicious yeast suspension (1.25g yeast/ml water). On the patch were eight 2nd instar *Drosophila melanogaster* larvae (instead of *D. subobscura*, which was used in Roitberg et al. (2010)), which were allowed to crawl on the patch for approximately 20 minutes prior to the experiment to guarantee the presence of a sufficient amount of host-derived cues, so-called “kairomones” (Vet, 1985). Females were allowed to forage freely until the danger cue by a puff of formic acid was delivered at a predetermined time. Puffs were generally delivered after 1, 5, 10, 15 or 20 minutes, but only if the female was actually searching on patch by that time. In cases where the wasp was off the patch or in the process of ovipositing, the danger cue was delayed until searching behaviour was resumed. The puff apparatus consisted of an Erlenmeyer flask filled with cotton wool up to approximately one centimetre, which was moistened with 500 µl of formic acid and replaced daily. The flask was closed with a rubber stopper with two holes for insertion of two PVC tubes (40 cm each). One of the tubes led to a syringe, the other to a 10 ml pipette tip. The syringe was drawn up to a volume of 20 ml. By pushing this volume out of the syringe, the airstream was directed through the flask with the saturated formic acid air and finally this airstream was routed through the second tube, which led to a short and defined puff approximately 7 cm above the searching parasitoid.

Once the danger cue was presented, the experiment was terminated. Patch abandonment within two seconds of the presentation of the danger cue was scored as a leaving response, otherwise it was scored as remaining on the patch. Apart from the response, other behavioural parameters such as position of the searching wasp and ovipositions were observed throughout the experiment using the software *The Observer XT* (Noldus Information Technology, 2008).

4.3.2 Pre-experimental treatment

Wasps and flies are reared as described elsewhere (Hoffmeister & Rohlfs, 2001). Females were separated within 24 hrs of hatching and placed into small glass vials containing an agar layer and a droplet of honey together with two males to ensure mating prior to the experiment. After another two days, the females were transferred into Eppendorff cups containing one of the three feeding substances (see below). 24 hrs before the actual experiment, wasps were given a first searching experience on a patch comparable to the one offered in the experiment, after which they were transferred back into their Eppendorff cup. Only wasps displaying searching behaviour on this pre-experimental patch were selected for the experiment.

4.3.3 Feeding treatment

Feeding instead of injecting neuroactive substances is an alternative with minimal stress for the animals. Several studies have already demonstrated that orally administered OA (Barron et al., 2007a; Schulz & Robinson, 2001) as well as DA (Sasaki et al., 2009) and the DA precursor DOPA (Harris & Woodring, 1999) lead to elevated brain titres of these substances.

Animals were fed *ad libitum* from 24 hrs up to 72 hrs with either a 20% sucrose solution (control) or the same solution containing 1.6 mM DA-hydrochloride or OA hydrochloride (both from Sigma Aldrich, Germany) respectively, which is comparable to the most effective dosages in feeding attempts in bees (Agarwal et al., 2011; Scheiner et al., 2002). Even though ascorbic acid had been added in other studies (Scheiner et al., 2002) to prevent oxidation, this test refrained from that, as observations of feeding behaviour revealed an avoidance behaviour towards acidic acid in *Asobara tabida*. All solutions were produced freshly on a weekly basis. Mortality prior to the experiment was low in both the control (2.6%) and the OA-treated group (1.65%) and although slightly higher in the DA-group (9.9%), still acceptable.

4.3.4 Data analysis

Statistical analysis was conducted using generalised linear mixed models in R 3.1.1 (R-Core-Team, 2014) using the lme4 library (Bates et al., 2013) with *observer* as a random term to correct for observer-specific differences. The optimal model was found using a stepwise backwards elimination of insignificant terms.

The first step analysed the influence of age (ranging from 3 to 7 days), time of the presentation of the puff of formic acid (ranging from 1 to 20 min) and drug treatment on the proportion of wasps leaving the patch measured as a binomial response with leaving = 1 and remaining = 0. As all variables were found to affect the wasps' response significantly, the data set was split by drug treatment in a second step for further analysis and plotting of the model.

4.4 Results

As expected, the time of the presentation of the danger cue affects the response. However, in the full model, this is only retrieved in interaction with the wasp's age (AGE X DANGER: X_1^2= 5.91, P=0.02, see Table 4-1, see Figure 4-1). Additionally, the response is strongly affected by drug treatment (X_2^2= 11.21, P=0.004, see Table 4-1, see Figure 4-1). The significant effect of drug treatment is only due to the effect of OA treatment (p= 0.001, see Figure 4-1), whereas the shape of the relationship reveals itself not to be significantly different when comparing the CONTROL and the DA treatment (p= 0.295, see Figure 4-1), even though qualitatively the chance to leave seems to be slightly higher in the DA compared with the CONTROL group. This is met in young wasps (percentage leaving at age of 3 days in DA: 60-66% [range of model

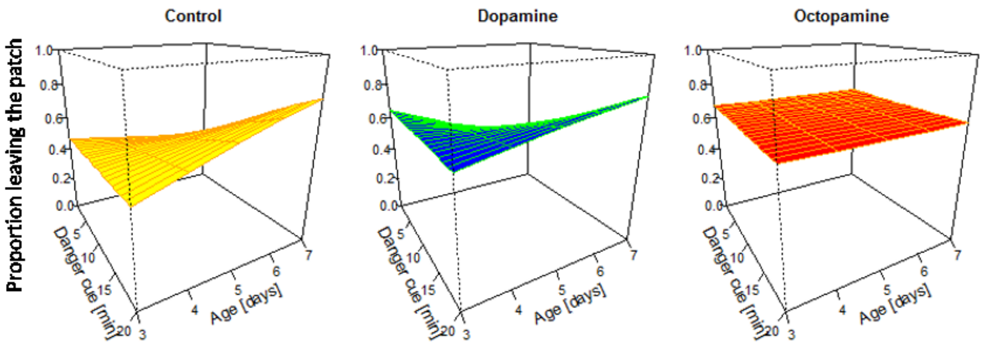

Figure 4-1: The proportion of wasps leaving the patch in response to the danger cue as a function of age [days] and time of presentation of the danger cue [min] during the patch visit. Plotted are the planes of best fit resulting from separate analyses for each drug treatment. Sample size: CONTROL = 239, DA = 248, OA = 210).

SE], in CONTROL 46-54%) as well as in older wasps (percentage leaving at age of 7 days over time of presentation of the danger cue in DA: 41-84%, in CONTROL 23-75%). In DA- and CONTROL-treated animals, the strong increase in the proportion of wasps leaving becomes more evident with age, while younger wasps are relatively indifferent towards the time of the presentation of the danger cue. Interestingly, the pattern is totally different in the OA-treated group, which becomes particularly evident if the data are analysed seperately for each drug treatment (see Table 4-2). While in CONTROL- and DA-treated animals all the variables affect the leaving response (although the effect of age alone is slightly insignificant in the CONTROL, see Table 4-2), in the case of OA treatment, none of the chosen variables is able to explain the proportion of wasps leaving the patch and the minimum adequate model equals the grand mean with a proportion of wasps leaving lying between 63.58% and 71.32% (based on the limits of the model's SE, see Table 4-2)

Table 4-1: Analysis of variance for the effects of the time of the presentation of the danger cue, age of the female and drug treatment as well as all possible interactions on the proportion of females leaving the patch in the case of perceiving a danger cue. Variables significantly affecting the leaving response bold printed. Non-significant terms being part of a significant interaction remain in the final model.

	Leaving response [%]
Time of presentation [min]	X_1^2= 1.54, p= 0.22
Drug treatment	**X_2^2=11.21, p= 0.003**
Age [days]	X_1^2= 2.44, p= 0.12
T_presentation*Drug treatment	X_2^2= 0.68, p= 0.71
Drug-treatment*Age	X_2^2= 0.74, p= 0.69
Age*T_presentation	**X_1^2= 5.91, p= 0.02**
T_presentation*Age*Drug treatment	X_2^2=4.65, p= 0.1

Table 4-2: Analysis of variance within each drug treatment for the effects of the time of the presentation of the danger cue and age of the female as well as their interaction on the proportion of females leaving the patch in case of perceiving a danger cue. Significant p-values bold printed. Non-significant terms being part of a significant interaction remain in the final model.

Leaving Response (binomial)	Control [N=239]	Dopamine [N=248]	Octopamine [N=210]
Time of presentation	**X_1^2= 9.27, P= 0.01**	**X_1^2= 9.71, P= 0.007**	X_1^2= 0.89, P= 0.34
Age	X_1^2= 4.96, P= 0.08	**X_1^2= 6.49, P= 0.04**	X_1^2= 0.02, P= 0.88
Time of presentation* Age	**X_1^2= 4.58, P= 0.03**	**X_1^2= 6.48, P= 0.01**	X_1^2= 0.32, P= 0.57

4.5 Discussion

This study used the experimental set-up established by Roitberg et al. (2010). This set-up makes it possible to test a behavioural trade-off, which requires balancing positive and aversive stimuli in order to decide optimally. This test changed the state of the wasps with respect to two parameters. Firstly, it employed wasps of various ages, as younger wasps should have different expectations regarding their future oviposition opportunities compared with older wasps (Liu et al., 2009) and are known to have shorter patch times even when they have had faced otherwise similar environmental conditions (Thiel et al., 2006; Thiel & Hoffmeister, 2004). Secondly, it manipulated intrinsic concentrations of the neuromodulators DA and OA, which are known to be fundamental in stimulus perception and responsiveness (Barron et al., 2010; Perry & Barron, 2013). Within the CONTROL treatment, the results corresponded largely to the pattern found by Roitberg et al. (2010) and the proportion of wasps fleeing the patch in the case of the presentation of the danger cue increased strongly over time (see Figure 4-1). Nevertheless, the ability to adapt a decision quickly to patch quality seems to be age-dependent and becomes more pronounced with age. Generally, it was expected that older wasps would respond later to the irritation compared with younger wasps. The older wasps have a different pre-patch experience with a lower average patch encounter rate and a decreased life expectancy and it could be shown that many parasitoids (Liu et al., 2009; Thiel et al., 2006; Wajnberg, 2006) but see (Jenner et al., 2012) and especially *Asobara tabida* (Thiel & Hoffmeister, 2004) respond with longer patch residence times with increased age. This may be proximately explained by an age-dependent increase in sensitivity, as has been found in bees (Behrends & Scheiner, 2012). Accordingly, young wasps should be more willing to leave compared with older conspecifics. However, that was not found. In fact, younger wasps did not noticeably adapt their decision to the rate of exploitation. 46-54% of the young wasps in the CONTROL group and 60-66% of the DA-treated young wasps left the patch irrespective of the time of the presentation of the puff. This does not mean that younger wasps do not perceive the cue as such, so that we can exclude a general lack of sensitivity, but rather that their decision concerning what to do is driven by chance or that they “flip a coin before deciding” (Plantegenest et al., 2004). As the authors point out, drawing one choice from a number of possibilities at random may be the best option if the outcome of the choices to choose from is uncertain. Young females experienced nearly one potentially stressful and unpredictable handling step caused by pre-experimental treatment per day, while older ones had a couple of days without any impact from the investigator. In light of this, it may be hard to assess the outcome of any decision, especially for younger wasps.

With regard to the drug treatment, a fundamentally different pattern was determined in OA-treated animals, whereas DA treatment did not cause any significant differences. The OA-treated animals not only had the highest rate of leaving on average (ranging from 63.58 to 71.32%), but also showed no adaptation in their decision relative either to their age or the time of the presentation of the puff. Basically, this was the opposite of what was expected. It was expected that OA-treated animals would be more willing to remain on patch, as it was assumed that their responsiveness towards the patch would be higher. The effect of octopaminergic treatment on the outcome of the fight or flight trade-off within a context of intrasexual competition has been extensively studied in crickets in the group around Schildberger (Rillich et al., 2011; Stevenson et al., 2005; Stevenson & Rillich, 2012) as well as in flies (Hoyer et al., 2008; Zhou et al., 2008), all showing a decreased likelihood of defending a given resource in the case of OA depletion and the authors mostly concluded that the effect of OA in aggressive behaviour was related to its role in the assessment of the reward value (Rillich et al., 2011; Stevenson & Rillich, 2012). However, those experiments were restricted to intrasexual competition. In contrast, increased levels of OA in crickets increased shelter seeking and general evasive behaviour similar to what was found in this experiment upon the arrival of a mimicked predator (Adamo et al., 2013). Furthermore, OA injection decreased the likelihood of being caught by a real predator caused by a fast flight response (Adamo et al., 2013). The present results head in the same direction, as OA-treated animals were more likely to leave the patch, which would probably decrease the risk of being caught if the predator were real.

It should be noted that most of the studies testing the effects of OA on responsiveness used nutritional rewards (Barron et al., 2007b; Barron et al., 2002; Scheiner et al., 2002; Scheiner et al., 2014) but see (Erber & Kloppenburg, 1995), which has actually since been criticised (Perry & Barron, 2013). The present study indicates that the effects found with respect to responsiveness cannot simply be transferred to different contexts. The link between the results in light of intrasexual competition, evasive behaviour and sugar responsiveness might be OA's role as a stress hormone (Roeder, 2005). Stressful stimuli like encounters with rivals or predators cause an increase in OA in the haemolymph and activation of energy reserves (Adamo & Baker, 2011; Roeder, 2005), preparing the animal for times of increased activity (Adamo et al., 1995), which is in general not necessarily related to a specific kind of activity. In this light, the high sensitivity to sucrose may simply be related to the higher metabolic rate under OA leading to higher energetic demands. Correspondingly, it has been shown in the blowfly *Phormia regina* (Long & Murdock, 1983) as well as in nymphs of the cockroach *Rhyparobia madera* (Cohen et al., 2002) that artificially raised OA titres caused increased feeding activity.

Stress strongly impacts the accuracy of decision-making processes, at least in humans (Starcke & Brand, 2012). Moderate levels of stress may actually increase the decision-making performance, probably due to an increased alertness, whereas increasing the stress level leads to suboptimal outcomes of decision-making processes, for example, because not all alternatives are scanned in stressful situations before deciding or because participants show impairments in the learning of cues that are relevant to make the optimal decision. Conflicting results exist concerning risk-prone or risk-averse behaviour (Starcke & Brand, 2012), although it mostly promotes risk aversion based on the review to which reference is made here.

The impact of stress on decision-making in humans basically reflects what was found in the case of OA treatment, namely an increased likelihood of leaving the patch in the case of the danger cue and no integration of patch quality. Therefore, it may be cautiously concluded that, at least in the set-up used here, OA acts as a stress hormone rather than as a modulator for stimulus specific responsiveness.

DA feeding did not cause significant behavioural modulations. However, it remains unclear if DA was not absorbed through the gut, if increasing DA artificially may not be useful to change behaviour or if the increase in intrinsic concentrations of DA was too slight to induce a behavioural change. Studies into DA ingestion in other organisms reveal conflicting results. No effects on behaviour at all were found in bees (Harris & Woodring, 1999; Scheiner et al., 2002), and while Scheiner et al. (2002) suggested that rapid oxidation of DA in the gut would prevent absorption, enhanced brain titres of DA and DOPA due to oral uptake were found (Harris & Woodring, 1999; Sasaki et al., 2009), which were irrespective of behavioural changes in former study. Nevertheless, finding enhanced brain titres of a given substance does not necessarily mean that the substance is present in the brain area where they should induce modulatory effects (Scheiner et al., 2006) and this may hold especially true for DA, as this substance is much more concentrated in specific brain areas compared to the more widely found OA (Menzel et al., 1999). Additionally, it could be that the amount of ingested DA was not sufficient to alter behaviour, as DA concentration is meant to be, at least in the bee brain, 2-3 times higher compared with OA (Harris & Woodring, 1992; Roeder, 1994). Nevertheless, in *Drosophila* it is the other way around (Hardie & Hirsh, 2006). However, it could still be feasible that lower amounts of OA are sufficient to alter behaviour if the intrinsic concentration is far lower while the relative additive effect of DA to the intrinsic level is negligible. Still, as results between species, within one group (Hardie & Hirsh, 2006) and even based on physiological factors within one species (Harris & Woodring, 1992) differ widely, the intrinsic concentrations and the uptake of biogenic amines in *Asobara tabida* still need to be tested.

This study was able to show that insect parasitoids serve as an optimal study system for questions regarding modulations in behaviour. This is, first and foremost, due to the detailed theoretical knowledge of optimal behaviour (Wajnberg, 2006) which enables researchers to address expectations regarding the optimal behaviour within a given situation clearly and a range of empirical studies supporting the theory (Godfray, 1994; Liu et al., 2009; Wajnberg, 2006). Additionally, their stereotype behaviour (Godfray, 1994), the close link between searching success and fitness (Godfray, 1994) that circumvents problems like decreasing motivation due to saturation and the possibility of combining different stimuli in time-dependent set-ups underline their ideal aptitude for studies of neuronal mechanisms in the field of optimal foraging. In light of the role of OA in responsiveness and reward assessment, parasitoid's host searching behaviour may offer an excellent complement when it comes to generalisations of the findings related to nutritional reward to reward assessment in general.

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Chapter 5

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The effects of biogenic amines on intrasexual competition and longevity in female Asobara tabida

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5.1 Abstract

Animals have to adapt their behaviour upon encounter of a potential competitor. Here the study tests the behavioural responses of the parasitoid wasp *Asobara tabida* upon arrival and behaviour of a competitor on a patch containing a moderate number of host larvae. Additionally, some of the wasps were fed either octopamine hydrochloride or dopamine hydrochloride as both substances have been shown to be important modulators in competitive behaviour in other arthropods, potentially via their role in reward and punishment assessment. Lastly, it analysed effects of ongoing competition for hosts and amine feeding on survival. Physical contacts between the competitors were found to be the only variable significantly and negatively affecting the tendency to stay on the patch. All other variables (ovipositions, ovipositions of the competitor, mere arrival of the competitor and food) did not cause any significant effects based on Cox regression analysis. Arrival of the competitor on the patch caused a significant increase in oviposition activity, indicating a higher likelihood to superparasitize. However, larvae were not parasitized at random, as efficiency decreased over time, even with a competitor present. Animals initiated more excursions over time and in the presence of a competitor, underlining that the animals were severely disturbed by the competitor's presence.

Host access diminished survival chances tremendously, while wasp density had a negative impact only in the absence of hosts, whereas the reverse was found if hosts were present. This may most likely be explained by restricted access to the hosts in inferior animals in the presence of competitors. Amine feeding did not have any effects on either behaviour or survival, with some slight effects on the latency to enter the patch being the only exception. As at least feeding octopamine is a well-established approach in other species, it can be concluded that the results question the generality of octopamine modulating intra-specific behaviour. Some potential reasons are discussed below.

5.2 Introduction

While animals searching alone can allocate their patch time based on the marginal value theorem (MVT) (Charnov, 1976), they cannot continue to do so once a competitor arrives (Haccou & van Alphen, 2008; Sjerps & Haccou, 1994; Wajnberg et al., 2004). The MVT predicts that a patch should be abandoned in the moment when the instantaneous gain rate drops below the habitat's average and the animals' ability to behave at least qualitatively in accordance to the MVT has often been shown, (see for example: Stephens & Krebs, 1986; Thiel & Hoffmeister, 2004; 2009; van Alphen et al., 2003; Wajnberg, 2006). However, a competitor's arrival changes basically everything: (1) It will dynamically change the instantaneous gain rate as a result of its own searching activity. (2) It may force the resident subject to reallocate time and energy. Whereas, as long as searching alone, the animal could invest time and energy exclusively in host searching activity, it may now also have to invest in patch defence and behavioural interactions with the conspecific. (3) Insect parasitoids face the problem that hosts, although parasitized, remain on the patch. Superparasitism by the conspecific may decrease their own offspring's chances of survival and, additionally, the animal has to decide if it should superparasitize itself or not. These reasons make the optimal allocation of patch residence time more complicated and less predictable and the variability in patch residence time increases (Haccou & van Alphen, 2008; Wajnberg et al., 2004).

Only very few studies focused on detailed observations of the behavioural responses of insect parasitoids to the presence and behaviour of potential competitors (but see Castelo et al., 2003; Wajnberg et al., 2004), and even less is known about the proximate mechanisms enabling the animals to reallocate patch residence times quickly in response to changing environmental conditions like the arrival of a competitor. Yet, for a single searching individual, it has been proposed that some internal mechanism might be involved in patch leaving decision-making, probably via the regulation of some internal thresholds modulated by some kind of "neurosecretory material" (Thiel, 2011), and it is likely that similar mechanisms should work irrespectively of whether the animal is searching alone or in a group.

The biogenic amines dopamine [DA] and octopamine [OA] appear to be suitable candidates for this "neursoscretory material". While DA has been proposed to be the key substance in aversive learning paradigms in invertebrates (fruit flies: (Schwaerzel et al., 2003); crickets: (Mizunami et al., 2009; Unoki et al., 2005; Unoki et al., 2006); honeybees: (Vergoz et al., 2007), this view is questioned nowadays (Perry & Barron, 2013; van Swinderen & Andretic, 2011), and a more complex role in task-specific attention and distractibility is coming into focus (van Swinderen & Andretic, 2011), which makes it a promising substance in patch-leaving behaviour. On the other hand, OA appears to be crucial in setting response thresholds predominantly towards rewarding stimuli (Barron et al., 2002; Behrends & Scheiner, 2012; Pankiw & Page, 2003; Scheiner et al., 2002; Scheiner et al., 2014; Schulz et al., 2002), which may be the prerequisite for the observed increased learning abilities caused by OA treatment (Hammer & Menzel, 1998; Mizunami & Matsumoto, 2010; Roeder, 1999; Unoki et al., 2005; Unoki et al., 2006). Furthermore, the role of OA in reward perception has been considered to be crucial for the observed effects on aggressiveness (Rillich & Stevenson, 2011; Stevenson et al., 2005), as the competitive effort invested should be relative to the expected gain from that action (Arnott & Elwood, 2008). Interestingly, it has been found just recently that DA also has a function in competitive behaviour, with DA-lacking inferior crickets being more likely to fight, perhaps because they lack the ability to integrate the aversive experience of being defeated (Rillich & Stevenson, 2014).

Here, the test investigates the response of *Asobara tabida* towards the arrival and behaviour of a competitor on a patch with a depletable number of hosts. Additionally, wasps had either DA or OA added to their diet as both substances appear to be key components in setting the level of responsiveness and in task-specific attention. Feeding of OA is a well-established and minimally invasive approach for manipulating behaviour (Barron et al., 2007; Scheiner et al., 2002; Schulz et al., 2002; Schulz & Robinson, 2001; Szczuka & Godzinska, 2008), whereas results for orally applicated DA are not presently that clear cut. While it has been shown to alter DA brain levels and to affect behaviour in some studies (Agarwal et al., 2011; Sasaki et al., 2009), it did not cause any effects in others (Nathanson et al., 1993; Scheiner et al., 2002). Firstly, the test analysed whether amine treatment and the behaviour of the competitor affects the tendency to enter the patch, i.e., latency. In general, latency should decrease with the predictability of a task (Papaj & Vet, 1990; Perez-Maluf & Kaiser, 1998; Perez-Maluf et al., 2008; Vet & Papaj, 1992), and may therefore serve as a proxy for learning rate, as all animals should be familiar with the experimental set-up, as they were all allowed to search within the same circumstances, albeit without a competitor the day prior to the experiment. Basically, OA-treated animals were expected to have a higher tendency to enter the patch due to the role of OA in lowering response thresholds. Furthermore, it is reasonable that the presence of a competitor on patch may lower the motivation to initiate searching activity, as the expected gain should be lower. However, to the knowledge of the authors, it has yet to be investigated whether *A.*

tabida avoids patches with conspecifics after identifying them from a distance, although other parasitoid species appear to do so (Castelo et al., 2003; Janssen et al., 1995b; Tamo et al., 2006), but see (Liu et al., 2009). Secondly, the test investigated which factors affected the tendency to stay on a patch, i.e., patch residence time. Additionally, it compared the number of excursions over time as an additional proxy for searching motivation and responsiveness to the patch (Thiel, 2011; Waage, 1979), thereby considering the arrival of the competitor as well as feeding treatment. It was assumed that OA-treated animals should have fewer excursions as they should be more responsive and that the presence of the competitor should lead to an increase in the number of excursions initiated. The oviposition efficiency was analysed as was a function of the presence of the competitor and food. While a clearly decreased efficiency was expected for trials without a competitor arriving, this effect should vanish when the competitor is present due to increased superparasitism (van Alphen & Visser, 1990; van Alphen et al., 1992; Visser et al., 1992). As OA-treated animals should have a lower response threshold, they should more readily accept already parasitized and therefore less valuable hosts.

Finally, the test measured the survival chances of animals receiving either food. To discover whether the potential effects of amine treatment were caused by a general sublethal toxicity, by effects on competitive behaviour or by modulations in host searching behaviour, they were kept either alone or in groups of ten and with or without *ad libitum* access to oviposition opportunities.

5.3 Material and Methods

5.3.1 Behavioural observations

Young female wasps of *Asobara tabida* (Nees) (Hymenoptera: Braconidae) were collected from their breeding tube within 24 hrs of hatching and kept isolated in small glass vials filled up to 1 cm with agar and capped with a foam plug at 20°C and in a 16/8 light/dark cycle until the experiment. The wasps used here were caught in Kiel, Germany, and kept in the laboratory for approximately 200 generations before the experiments took place. The wasps in the experiment were 13-14 days old. This corresponds to roughly 1/3 of lifetime in the lab and in the absence of competitors (own observation). *A. tabida* is moderately synovigenic. They hatch with an average egg supply of approximately 150 eggs (Le Lann et al., 2014) and may produce up to approximately 600 eggs in their lifetime (Jervis et al., 2008)

5.3.1.1 Feeding treatment

Dopamine hydrochloride and octopamine hydrochloride were purchased from Sigma-Aldrich, Germany. For both drug treatments, 0.62mg/g of either substance were dissolved in honey; pure honey served as control. Honey solutions were placed on the foam plug in *ad lib* amounts and replaced weekly.

5.3.1.2 Experimental set up

A Petri dish measuring 60 mm in diameter served as the experimental arena. Petri dishes were lined with agar and a small patch of baker's yeast measuring 2 cm in diameter located in the centre, with eight early 2nd instar larvae of *D. melanogaster* crawling on it. All females experienced a second patch of the same quality one day prior to the experiment. Only wasps showing normal searching behaviour on this pre-experimental patch were selected for the experiment.

Both wasps were introduced into the arena in quick succession. The behaviour of both individuals was scored using the software The Observer XT 3.0 (Noldus-Information-Technology, 2008). Scores were awarded for the occurrence and duration of all relevant behaviours such as: walking, preening, resting, detecting a larva, oviposition, wing fanning and physical contact as well as the position (being on or beyond the patch) for both animals. Each observation was terminated after 40 min.

5.3.1.3 Statistical analysis

All analyses were conducted using the statistical software R.3.2.1 (R-Core-Team, 2014).

Firstly, an analysis was performed of the effect of FOOD and ARRIVAL of the competitor on the patch on the tendency to enter the patch (LATENCY). This analysis was restricted to the first wasp introduced in order to avoid data dependencies. Subsequently, the effect of ARRIVAL of the competitor, physical CONTACT with the conspecific, OVIPOSITION events of both individuals and FOOD on the tendency to leave the patch again was analysed. Consequently, all data for the first (and sometimes only for the first) wasp arriving on patch were taken. Both analyses were conducted using time-dependent Cox regression (Therneau, 2014), with FOOD as a fixed, ARRIVAL of the competitor and, in the case of the second analysis only, physical CONTACT, OVIPOSITION and ovipositions of the competitor (OVICOMP) as time-dependent variables. Patch residence time [PRT] was defined as the time from the first entering of the patch up to the time when the animal left the patch for more than 30 seconds.

Models were checked for proportionality of the effects based on Schoenfeldt residuals (Therneau, 2014).

Secondly, GLM analysis (Fox & Weisberg, 2011) was conducted to test if the duration of physical contacts differs depending on FOOD. The duration of physical contacts was best analysed using a Gamma distribution with log-link function.

Finally, each trial was divided into three fractions of the same length and behaviour within the 1/3 and 3/3 part of the observation was compared by the means of geeglm (Højsgaard, 2006) to correct for data dependencies, as each observation contributed 2 data points to this analysis. The number of physical contacts and the number of oviposition attempts and excursions from the patch were calculated for each third. These data were subsequently divided by the length of the third to correct for intra-observational differences. The data achieved were all right skewed and contained zeros. A square root transformation led to approximate normal distribution. For a graphical representation of the results, the predicted values were back-transformed. Bar graphs were created using the *gplots* library (Warnes et al., 2015).

5.3.2 Effects of intrasexual competition and aminergic treatment on longevity

5.3.2.1 General protocol

Drug treatment corresponded to the one described above.

Initially, survival was measured for all the wasps in the experiments. To do so, all the wasps were put back into their glass vials after the experiment, FOOD was replaced weekly and humidity was kept constantlyhigh by replacing the agar when it dried out. FOOD-dependent effects on longevity were analysed using the *survival* library (Therneau, 2014).

The second survival experiment was set up to estimate the effects of competition and amine feeding on longevity. This was done by taking 2-sided, open tubes measuring 2.5 cm in diameter and 6 cm in length, which were plugged on top with a foam plug moistened with honey solution of either FOOD treatment and with little plastic cups filled up to 3 mm with fly-rearing medium on the bottom. As the diameter of the plastic cup reduced slightly towards the bottom the plastic tubes, they were plugged nicely approximately 2 mm above the medium. The fly-rearing medium either contained an unrestricted number of initially two-day-old late 1st and early 2nd instar larvae of

Drosophila melanogaster or was uninfected. The densities were either one wasp or 10 wasps initially put into the vial.

To approximate the number of fly larvae between the tubes, cups with the rearing medium were placed in fly-rearing cages for roughly one hour, depending on the flies' egg-laying activity. Fly eggs were incubated for two days at 20°C before the cups were placed under the tube. Medium cups were replaced every second day. The number of dead individuals was noted during the replacement. Experimental wasps were kept at 22°C and in a 16/8 light/dark cycle.

5.3.2.2 Fly-rearing medium

The fly-rearing medium was composed of 12 g of agar dissolved in 500 ml water with 50 g of sugar stirred into a mix of 250 g of apple sauce, 100 ml of water, 70 g of brewer's yeast and 50 g of maize meal. 10 ml each of ethylparaben and sorbic acid dissolved in ethanol were added as preservatives.

5.3.2.3 Statistical analyses

Cox mixed effect models were fitted to the data using the *coxme* library (Therneau, 2012) in R.3.2.1. HOST availability, DENSITY and FOOD were considered as explanatory variables. TUBE was specified as a random term to correct for non-independence of datapoints coming from the same tube.

5.4 Results

5.4.1 Behavioural observations

5.4.1.1Tendency to enter the patch (Latency)

For this analysis, there were 60 data points available overall. Of those, 20 belonged to the CONTROL, 21 to the DA treatment and 19 to the OA treatment.

Latency was significantly and positively affected by arrival of the competitor on patch, which is expressed in the strongly decreased tendency to enter the patch in response to the arrival of the competitor (ARRIVAL competitor: N= 60, β=-1.06, exp(β)= 0.35, se(β)= 0.32, z= -3.34, $p < 0.001$). This effect was retrieved in all three feeding treatments as the interaction of FOOD X ARRIVAL is insignificant (χ^2=3.26, df= 2, p=0.20). However, in general, it took DA-treated animals longer to enter the patch (β=-0.73, exp(β)= 0.48, se(β)= 0.37, z= -1.97, p= 0.048), whereas OA treatment did not cause any effects (β=-0.02, exp(β)= 0.98, se(β)= 0.34, z=-0.07, p=0.94). The overall fit of the additive model was found to be X^2=13.52, df=3, p=0.004.

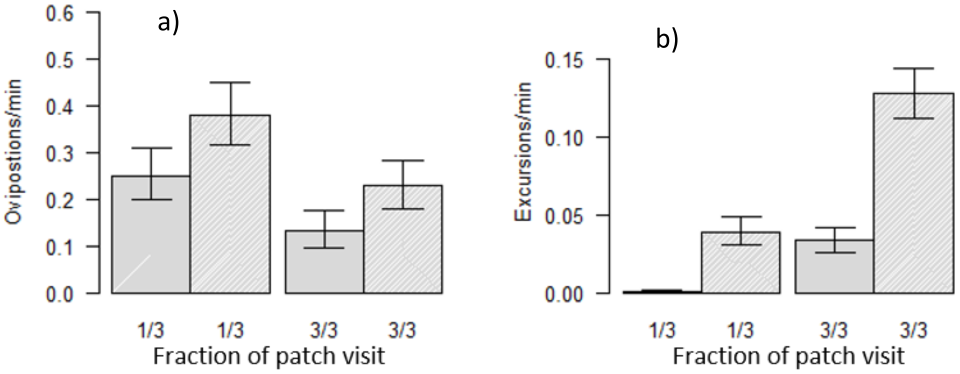
5.4.1.2 Tendency to stay on the patch (Patch residence time)

In three observations, none of the wasps entered the patch within the 40 minutes determined for each observation. Interestingly, all three belonged to the DA treatment, which reducesthe number of replicates down to 18 in this treatment.

The tendency to stay on the patch is only significantly affected by contact with the competitor (CONTACT: β= 0.20, exp(β)=1.22, se(β)= 0.08, z= 2.56, p= 0.01), meaning that each CONTACT decreases the tendency to stay by roughly 22%. FOOD does not have any significant effect either per se (FOOD: χ^2= 0.42, df= 2, p= 0.81) or in any interaction (FOOD X CONTACT: χ^2= 3.22, df= 2, p= 0.20, FOOD X OVIPOSITION: χ^2= 2.39, df= 2, p= 0.30, FOOD X ARRIVAL: χ^2= 0.02, df= 2, p= 0.99, FOOD X OVICOMP: χ^2= 0.67, df= 2, p= 0.71). Apart from the aforementioned effect of CONTACT, no other effect of the presence of the competitor was found. Neither the mere ARRIVAL (χ^2= 0.94, df= 1, p= 0.33) nor oviposition attempts by the competitor (OVICOMP χ^2= 0.94, df= 1, p= 0.33) affected the leaving tendency.

5.4.1.3 Behaviour over time

Oviposition efficiency decreased significantly from the 1/3 to the 3/3 fraction of the patch visit (THIRD: χ^2_{2X57}= 6.07, df= 1, p= 0.01, see Fig. 5-1a). This is what is expected as the patch suffers gradual depletion. Upon ARRIVAL of the competitor, the number of ovipositions attempts per minute increases significantly (ARRIVAL χ^2_{2X57}= 5.25, df= 1, p= 0.02) irrespective of the fraction (ARRIVAL X THIRD: χ^2_{2X57}= 0.62, df= 1, p= 0.43) as can be expected with an increased tendency to superparasitize. However, even in the presence of the competitor, the oviposition efficiency decreases over time, showing that superparasitism does not occur at random even under competition (see Fig. 5-1a). The number of excursions per minute increases significantly from the 1/3 to the 3/3 fraction of the patch visit (THIRD: χ^2_{2X57}= 3.33, df= 1, p= 3.2e-07) and with the arrival of the competitor as well (ARRIVAL χ^2_{2X57}= 10.5, df= 1, p= 0.001). Interestingly, the number of excursions within the 1/3 with a competitor arriving raises up to the level of the 3/3 in the absence of the competitor (see Fig. 5-1b). The likelihood of coming into physical contact increases steeply from the 1/3 to the 3/3 fraction of the patch visit (THIRD: χ^2_{2X57}= 24.4, df= 1, p= 7.8e-07, see Fig. 5-1c). It should be noted that this is not due merely to the increasing chance that the competitor arrives, as the same pattern is also retrieved if only the subset is analysed in which the competitor arrives within the 1/3 (THIRD χ^2_{2X34}= 7.29, df= 1, p= 0.007). In this case, the number of physical contacts rises from 0.03 (SEL= 0.01, SEU= 0.05) contacts/min in the 1/3 to 0.14 (SEL=0.01, SEU= 0.19) in the 3/3.



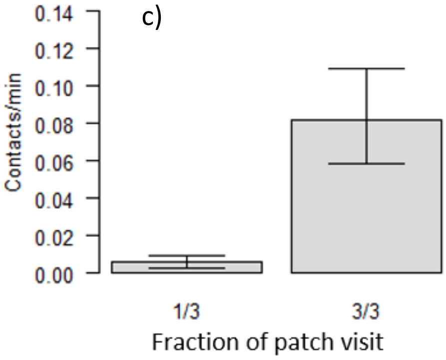


Figure 5-1: The rates of a) ovipositions, b) excursions and c) physical contacts expressed as events per minute within the 1/3 and 3/3 fraction of the patch visit of a female Asobara tabida wasp. Plotted are the predicted values from the minimal adequate model found by GEEGLM analyses. All dependent variables were square root transformed to achieve normal distribution for analysis. For better interpretability, the plotted values are back-transformed. Dashed bars refer to observations with a competitor present (Presence or absence as variable was not part of the last model, as the arrival of the competitor is a prerequisite for physical contacts). Only significant effects are shown here.

Food does not have any significant effect on any of the behavioural variables tested here (see Table 5-1).

Table 5-2: Results of GEEGLM analyses on the effects of FOOD on ovipositions/min, excursions/min and physical contacts/min.

	N	df	FOOD	FOOD X THIRD	FOOD X ARRIVAL
Ovipositions/min	2X57	2	χ^2=0.09, p= 0.95	χ^2= 1.23, p= 0.54	χ^2= 2.48, p= 0.29
Excursions/min	2X57	2	χ^2= 1.23, p= 0.54	χ^2= 0.60, p= 0.74	χ^2= 4.32, p= 0.12
Contacts/min	2X57	2	χ^2= 0.68, p= 0.71	χ^2= 0.40, p= 0.82	Not considered

5.4.2 Effects of aminergic treatment on longevity

5.4.2.1 Survival of the experimental wasps

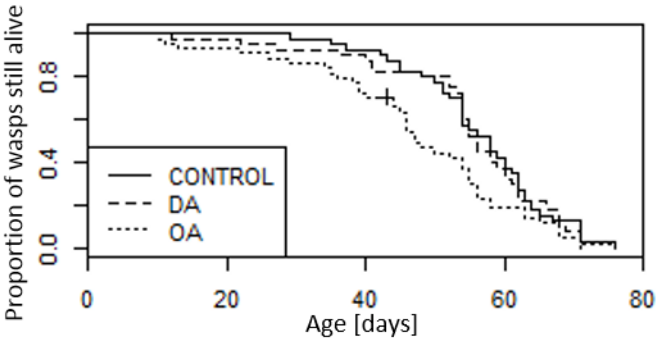


Figure 5-2: Kaplan-Meyer survival curves showing the proportion of experimental wasps still being alive at a given age [days].

With 58 days in CONTROL animals (N=40) and 56 days in DA-treated ones (N=40), median survival was only slightly higher compared to OA-treated individuals (47 days, N=44, see Fig. 5-2). However, this difference is slightly insignificant ($\chi^2 2= 4.4$, p= 0.09862).

5.4.2.2 Effects of intrasexual competition and aminergic treatment on longevity

The effect of HOST availability was highly significant ($\chi^2= 123.94$, df=2, $p< 2.2e-16$). In the absence of hosts, median survival was more than doubled compared to animals with ongoing oviposition opportunities (see Table 5-2, Fig. 5-3). Interestingly, a significant effect of wasp DENSITY was only found in interaction with HOST availability (DENSITY X HOST: $\chi^2= 9.86$, df=2, p= 0.002). This is explained by a reversed effect of DENSITY within the two HOST treatments. While in the absence of hosts survival is negatively affected by high DENSITIES (although this effect is qualitatively not retrieved in OA-treated animals, see Table 5-2, Fig. 5-3), the opposite is actually found if animals have had host access. In this case, median survival was consistently higher if animals were kept in groups compared to conspecifics kept alone (see Table 5-2, Fig. 5-3).

FOOD has had no significant impact either per se (FOOD: $\chi^2=1.34$, df= 1, p=0.51) or in interaction with HOST availability ($\chi^2=2.73$, df= 2, p=0.26) or DENSITY ($\chi^2=2.60$, df= 2, p=0.27, see Fig. 5-3).

Table 5-3: Median survival split by HOST availability, wasp DENSITY and FOOD

		CONTROL		DA		OA	
Density	N	Host	No host	Host	No host	Host	No host
1	10	16	46	13	47	14	40
10	3*10	18	39	18	39	16	43

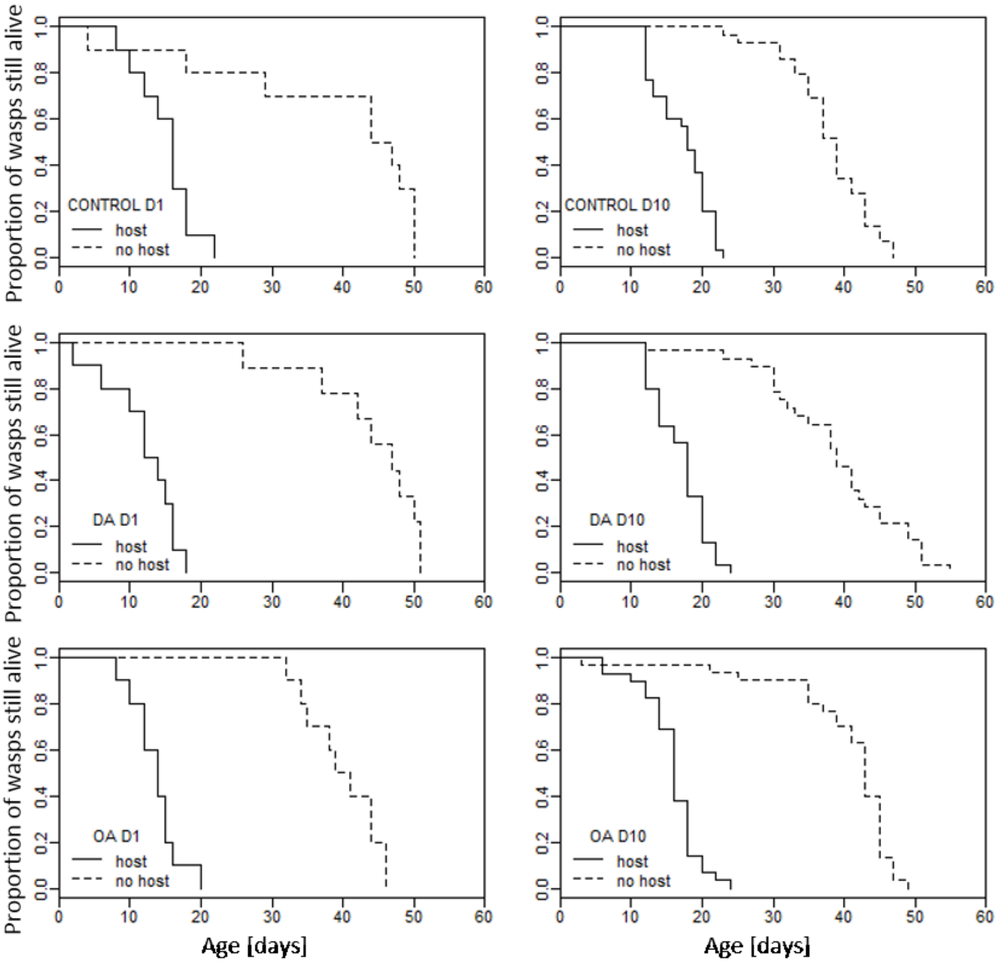


Figure 5-3: Kaplan Meyer survival curves describing the proportion of individuals alive at a given age. Separate panels are given for different feeding treatments and wasp densities, from top to bottom: Control, DA, OA with a density of 1 individual [D1] left or 10 individuals [D10] right. Solid lines: wasps had access to hosts ad libitum, dashed lines: wasps had no access to hosts.

5.5 Discussion

The aim of this work was to investigate the effects of arrival of a conspecific on behaviour and patch leaving decisions in *A. tabida*. Special emphasis was placed on intraspecific behaviour. Additionally, some of the animals were administered octopamine hydrochloride or dopamine hydrochloride orally, as both substances are known to modulate competitive and task-related behaviour (Dierick, 2008; Rillich et al., 2011; Stevenson et al., 2005; Stevenson et al., 2000; Stevenson & Schildberger, 2013; van Swinderen & Andretic, 2011). Lastly, survival was tested under varying regimes of competition, host availability and amine treatment.

Up to now, competitor-caused effects on behaviour in *A. tabida* were only found to lead to an increased likelihood to superparasitize (de Jong et al., 2011; Hemerik et al., 2002; van Alphen et al., 1992), whereas the effects of physical contacts and the mere presence of a competitor on behaviour have never been shown in that species so far. However, it has been pointed out that patch defence and direct competition only serve as an adaptive strategy if patches are rather small and host numbers are low (van Alphen & Visser, 1990). In other species, it has been shown that individuals adjust behaviour appropriately to patch size and host density (Waage, 1982). Here, it was found that individuals react strongly to physical contacts with the competitor and each encounter reduces the tendency to remain on the patch. Although this has been shown in other species (Wajnberg et al., 2004) for *A. tabida*, this has not been shown before (de Jong et al., 2011; Hemerik et al., 2002). This may be due to the chosen patch size here, which was considerably smaller than in the other experiments mentioned, or due to the lower number of hosts, both increasing the defendability of the resources. Furthermore, competition in the described experiment only took place between two individuals, facilitating the analysis of the effect of physical encounters. It should be pointed out that physical contacts remain as the only variable in the model explaining the tendency to stay on the patch. This is consistent with the results of Wajnberg et al. (2004) on *Trissolcus basalis* and also with the predictions formulated in Haccou et al. (2003) proposing that variability in patch residence time should increase if more than one individual is on the patch. As, for example, the outcome of an oviposition becomes less predictable, the effect of an oviposition with respect to the tendency to stay on the patch should become more variable too. Secondly, individuals even respond to the conspecifics behaviour before they actually encountered the patch, by reacting with a reduced tendency to enter the patch if it is already occupied by the competitor. At present, latency is mostly neglected in patch time allocation experiments (but see Papaj & Vet, 1990; Perez-Maluf & Kaiser, 1998; Perez-Maluf et al., 2008; Vet & Papaj, 1992). This is astonishing as the strong impact of efficiency on fitness is one of the main concepts in optimal patch time theory. Logically, efficiency decreases with increasing latency and therefore animals should enter quickly upon identifying the vicinity of a patch, especially if the predictability of the outcome of the patch visit is high (Perez-Maluf et al., 2008). The animals in this experiment were all familiar with the experiment and in general latency is highly reduced in experienced animals even after just one pre-experimental experience (Papaj & Vet, 1990; Perez-Maluf et al., 2008; Vet & Papaj, 1992), indicating that the strongly fitness-relevant experimental set-up in general is quickly learned. However, in the demonstrated experiment, the average latency was not reduced from the pre-experimental to the experimental patch. The predicted time needed to enter the pre-experimental patch based on survival analysis was CO: 5 min (LCL: 3, UCL: 20, N=52), DA: 13 min (LCL: 7, UCL: 23, N=49), OA: 4 min (LCL: 3, UCL: 12, N=48) and CO: 5 min (LCL: 3, UCL: 20, N=20), DA: 11 min (LCL: 2, UCL: NA, N=21), OA: 4 min (LCL: 3, UCL: 20, N=19) for the first wasp introduced to the experimental patch, which is evidently rather similar to the experimental patch. Cox regression analysis indicates that this still relatively long average latency on the experimental patch is at least in part explained by the patch encounter of the competitor. Logically, the response may be expected, as the quality of the patch and the predictability of the outcome decrease considerably with a competitor on patch. However, whether parasitoids are capable of such an anticipation of changes in outcome remains hypothetical, and actually, depending on the species, parasitoids react to the combination of cues deriving from competitors and hosts in patch choice experiments quite differently. Whereas, for example, *Venturia canescens* does not consider odour cues of competitors in decision-making (Liu et al., 2009) or does so only at very high densities (Castelo et al., 2003), *Cotesia marginiventris* and *Leptopilina heterotoma* have been shown to avoid landing on patches with a superior related species (Janssen et al., 1995a; Tamo et al., 2006) or conspecifics (Janssen et al., 1995b). The authors of this test were able to show that *A. tabida* is less likely to enter the patch if it is already occupied, which may be interpreted as an avoidance response towards conspecific females.

Simultaneous search affected behaviour considerably. Firstly, animals left the patch more frequently if a competitor was present and, secondly, they made more oviposition attempts per minute than in the absence of competitors. This can only be explained by a higher acceptance of already parasitized hosts as has previously been shown in other studies on *A. tabdia* (de Jong et al., 2011; Hemerik et al., 2002) as well as other endoparasitoids (van Alphen et al., 1992; Visser, 1993; 1995). The increase in the number of excursions per minute upon the presence of a competitor indicates that the searching animal is severely disturbed by the activity of the competitor and, although not explicitly analysed here, the authors observed that physical contacts frequently resulted in a distinct temporal flight from patch by one of the individuals, indicating that physical contacts do indeed have a competitive function in *A. tabida*. Finally, the number of physical contacts rises steeply from the 1/3 to the 3/3 even if only those observations with the competitor arriving in the 1/3 are considered. If physical contacts were only the result of an accidental trip over one another, then the chance of coming into physical contact should remain constant. However, this was not the case. On the other hand, that pattern also shows that patch defence is probably not the primary function of physical contacts. For instance, in the patch-defending related species *Asobara citri*, fights are readily initiated when competitors are encountered (de Jong et al., 2011). In this case, however, the first contact occurred only 4.5 min (SEL 4 min, SEU: 5 min) after the second animal has arrived on the patch.

In summary, it can be concluded that the presence of a competitor affects a searching female of *A. tabida* in numerous ways, all indicating that the sense of the competitor is a negative experience. The role of physical contacts on behaviour in that species has until now been neglected, but, although they are only punctual events, which may not be defined as real fights, they do have a strong modulatory effect on patch-leaving behaviour.

The impact of feeding DA or OA respectively is restricted to some minor modulatory effects of DA on latency. There may be different reasons for this: First of all, the effect may be related to a detrimental effect on motor ability as has been observed after injection of DA in honeybees (Mustard et al., 2010) and *Drosophila* (Yamamoto & Seto, 2014). However, as the authors did not find any food-related differences in behaviour that could indicate an effect on mobility caused by DA, e.g., a lowered oviposition efficiency should be expected if motor abilities are negatively affected, it can be concluded that this is very unlikely to be the reason for the lowered tendency to enter the patch. Secondly, the effect may be caused by a general response to the competitor. This can be excluded, as quantitatively the same response was found in response to the pre-experimental patch where no competitor was present (see above). Thirdly, DA feeding increases the response threshold to the volatile cues emitted by the patch. Increased response thresholds towards rewarding stimuli in the case of DA injection have been reported from other set-ups as well (Blenau & Erber, 1998; Scheiner et al., 2002). Still, behaviour on patch contradicts the hypothesis of increased response thresholds, as once on patch no differences were found that would indicate differences in response thresholds (for example: more frequent excursions from the patch). Thus, the role of DA on latency needs to be illuminated further.

The second experiment served to reveal whether potential effects of amine treatment on longevity were caused by a general sublethal toxicity, by effects on competitive behaviour or by modulations in host searching behaviour. An increased mortality rate in the case of a diet containing a high concentration of OA has been reported for ants (Szczuka & Godzinska, 2008), while detrimental effects of chronic DA treatment have been reported for rats (Benshachar et al., 1995) and humans (Sakr et al., 2006). However, the concentrations used here did not have a significant effect on survival, although the trend showed reduced chances of survival for OA-treated wasps found for the experimental wasps may indicate that increasing the concentration of OA might cause detrimental effects. Furthermore, amine-treatment did not cause significant interactions with density and/or the presence/absence of hosts. Actually, this is not surprising based on the results of the behavioural observations, as no indication was found that amine treatment would alter intraspecific or searching behaviour. Interestingly enough, however, density and the presence/absence of hosts interacted significantly. Initially, it has been hypothesized that density and host availability would affect survival in an additive way. It was assumed that the presence of competitors would be a potentially stressful experience that could be especially detrimental if hosts are present to compete for. It has now been revealed that only the first assumption was met. Although most research on density-dependent mortality focused on premature stages, detrimental effects of adult density on longevity have been found in numerous insect species (Greenberg et al., 1995; Lane & Mills, 2003; Paranhos et al., 2008; Peters & Barbosa, 1977; Zurlini & Robinson, 1980), although the results are not unambiguous (Brent, 2010; Peters & Barbosa, 1977). Zurlini and Robinson (1980) hypothesized that increasing density correlates with increasing physical contacts, which serve as a stressor forcing the animals (in that case onion flies) to move. Based on the authors' results and observations of *A. tabida*, they would support that hypothesis. Interestingly, host access has no amplifying effect on mortality in the animals facing high densities, with the opposite instead being the fact. Indeed, median survival was slightly longer if hosts and competitors were present compared with animals which did not have to deal with competitors. Three things can be concluded from the pattern found here: First, density is a stressor for *A. tabida* per se and does not need competition to occur, as in the absence of hosts there is nothing to compete for directly. This continuous stress leads to a slight detrimental effect on longevity. Second, the presence of hosts dramatically decreases longevity as predicted by the longevity-reproductive trade-off, which is especially distinct in most parasitoids, as they are unable to refill lipid reserves required for egg maturation and maintenance (Ellers, 1996; Ellers et al., 2000; Ellers & vanAlphen, 1997; Jervis et al., 2008; Visser & Ellers, 2008). Third, *A. tabida* competes for fitness not only via superparasitism, but also via a restriction in host access. If host access were not constrained, then we would actually expect either the same effect of host access on longevity irrespective of conspecific density or even a stronger detrimental effect caused by either additive effects of the stress due to the presence of the conspecifics or by an increased likelihood to superparasitize, accelerating consumption of energy reserves. The fact that the opposite has now been found can only be explained by a limited oviposition success due to the presence of conspecifics. As has already been shown within the analysis of the behavioural observations, animals leave the patch more frequently in the presence of a competitor. Additionally, physical contacts increase the leaving tendency significantly. In the set-up used here, there is a patch of approximately the same surface area but with ten individuals initially competing for the hosts. The number of physical contacts will likely increase with more competitors present and the animals are likely to leave the patch more often and make fewer oviposition attempts. This results in a decrease in the consumption of energy reserves and, accordingly, prolonged survival.

It is worthy of note that OA treatment did not cause any significant effects although oral OA administration is a well-established method in hymenopterans (Barron et al., 2007; Scheiner et al., 2002; Schulz et al., 2002; Schulz & Robinson, 2001; Szczuka & Godzinska, 2008). Although it might be the case that the concentrations used here are too low to elicit significant responses, this remains questionable. The concentration is actually based on the results in Scheiner et al. (2002), resulting in the highest effect size. The concentration used was corrected by the mean bodyweight of the parasitoids and actually doubled again after it was found that effects were rather subtle in previous experiments. As such, the conclusion is that the intake rate should generally be sufficient to elicit detectable responses, although uptake certainly needs to be tested. The results question the generality of the role of OA in reward modulation. Most of the studies into the role of OA in response thresholds and reward value have been conducted using sugar (Barron et al., 2007; Scheiner et al., 2002; Schulz et al., 2002; Schulz & Robinson, 2001) or other food rewards (Szczuka & Godzinska, 2008) cementing the theory of OA being the modulator of reward. A reduction in competitive behaviour in OA-depleted individuals has been consequently explained as well by OA's role in reward assessment (Rillich et al., 2011; Stevenson et al., 2005; Stevenson et al., 2000; Stevenson & Schildberger, 2013). However, it has long been known that OA is essential in the activation of the energy metabolism as well (Roeder, 2005) and, accordingly, reduced aggressiveness in OA-depleted animals might have nothing to do with reward modulation, but rather be the result of deficiencies in the activation of energetic reserves (Dierick, 2008). Following this theory consequently brings up the question if the reported effects of OA with respect to responsiveness to food might not be the simple result of a modulation in physiological needs rather than evidence for its general role in reward modulation. This might explain why no OA-related effects were found here.

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Chapter 6

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The effects of biogenic amines on intrasexual competition and longevity in males of the parasitic wasp Asobara tabida

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Contributions: Conceived and designed the study: JU. Data collection: JU. Analyzed the data: JU. Wrote the paper: JU.

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6.1 Abstract

Octopamine and dopamine have been shown to be important modulators in male competitive behaviour in flies and crickets. Amongst other things, this finding has been attributed to their role in reward and punishment assessment. This test investigated the effects of oral administration of either substance on the behaviour and longevity of male parasitic wasps of *Asobara tabida*. Physical contests occurred frequently and were of considerable duration irrespective of amine treatment. In contrast to what was expected, significantly fewer physical interactions were found in octopamine-treated animals; dopamine feeding has no effect on competitive behaviour, although a trend indicating increased walking activity in this group was evident. Amine treatment did not affect longevity in wasps held in isolation, but octopamine treatment did increase the risk of mortality risk if wasps were kept in groups. Furthermore, while the presence of females was the main factor increasing mortality in untreated males, although most likely via increased intrasexual competition, in both dopamine-treated and octopamine-treated animals, male density caused the strongest effects, with the effect of the presence of females being only slight but still evident.

6.2 Introduction

Aggressiveness, especially among males is very common throughout the animal kingdom (Arnott & Elwood, 2008). Interestingly, relatively little is known about aggressive behaviour in male parasitoids, although the evidence indicates that parasitoids in general do not form an exception (Godfray, 1994; van den Assem et al., 1980). It seems that some ecological circumstances promote the evolution of intra-male aggressiveness and one of the main promoters seems to be the simultaneous development of not too closely related individuals (Godfray, 1994) as may be the case in gregarious and in pseudo-gregarious species. In gregarious species, clutches of eggs are laid on or in the same host, whereas pseudo-gregarious species are usually solitary parasitoids which attack patchily distributed hosts (Hardy, 1992; van den Assem et al., 1980). Simultaneous development of numerous not necessarily related individuals is the consequence. However, while in truly gregarious species the sex ratio upon emergence can be highly biased towards females (Smart & Mayhew, 2009), a phenomenon which has also been noted in isolated females of the pseudo-gregarious *Drosophila* parasitoid *Leptopilina heterotoma* (Debout et al., 2002), in field studies on parasitoids of *drosophilids,* no such shifts in sex ratio, or only slight ones, have been found and males and females hatch at approximately the same rate (Carton et al., 1986; Fauvergue et al., 1999). Nevertheless, a balanced sex ratio promotes direct or indirect competition among males as they enhance their fitness mostly by maximising the number of matings, while female Hymenopteran parasitoids mate predominantly only once (Boulton et al., 2015; Ridley, 1993). Furthermore, if additional matings occur, the first male to mate fathers most of the offspring (Quicke, 1997). Evidently, under these circumstances females become a limited resource for males.

The solitary endoparasitoid of *Drosophila* larvae *Asobara tabida* is a pseudo-gregarious species. At least in the lab the sex ratio is relatively balanced. Male specimens emerge slightly earlier compared to the females (Carton et al., 1986; Stoekl et al., 2014; Thiel, 2004), which indicates that most matings take place right at the emergence site (Godfray, 1994; Hardy, 1994). Accordingly, males should be very likely to compete with each other in order to maximise mating success. However, to the best of the authors' knowledge, intra-male competitive behaviour has never been tested in detail for this species as male behaviour in general has been, although only recently a number of studies investigated male courtship behaviour in *A. tabida* (Dufour et al., 2012; Louapre et al., 2014; Stoekl et al., 2014).

In a number of studies on male drosophilids and crickets, it has been shown that the neuromodulators octopamine (OA) and dopamine (DA) are important substances in intra-male aggressive behaviour. OA-depleted animals are less likely to fight than untreated specimens (Baier et al., 2002; Dierick, 2008; Hoyer et al., 2008; Rillich et al., 2011; Rillich & Stevenson, 2011; Rillich & Stevenson, 2014; 2015; Zhou et al., 2008), but see (Momohara et al., 2013). It has been argued that the decreased likelihood to fight might be related to the role of OA in reward modulation (Rillich & Stevenson, 2011; Stevenson et al., 2005), but see (Dierick, 2008), as subjective reward value seems to correlate with intrinsic OA concentration (Barron et al., 2007). As the physical investment (including energy spent in fighting activity) should relate to the predicted outcome of that action (Arnott & Elwood, 2008), it seems to be logical that the likelihood of fighting correlates with intrinsic OA concentrations. More recently, a blocking of DA release has been shown to increase the likelihood of fighting. This finding was explained to be the result of a disability of the inferior animal to integrate the aversive experience of being defeated properly (Rillich & Stevenson, 2014).

This test analysed the behaviour of two *A. tabida* males, restricted from access to females and males for 12 days with respect to competitive behaviour. This time span was chosen as it has been assumed and shown that male investment in intersexual competition should follow the predictions of the MVT (Godfray, 1994; van den Assem et al., 1984). If females are rare, they should be more intensely courted and more readily accepted even if they are of minor quality (e.g.*,* already mated). It is assumed that limited access to females should also increase the tendency to fight between males.

Males are placed together into a Petri dish previously visited by a female. Odour cues elicited by the female have been shown to be sufficient to induce courtship behaviour (Stoekl et al., 2014) in *A. tabida* and have the advantage that no non-focus interactions may impede the analysis of male-male interactions. Furthermore, males were treated orally with either DA or OA dissolved in honey or kept on pure honey throughout their life. The authors decided on the minimally invasive approach of oral administration as it is a well-established method in OA treatment (Barron et al., 2007; Scheiner et al., 2002; Schulz et al., 2002; Schulz & Robinson, 2001; Szczuka & Godzinska, 2008), whereas conflicting results exist for DA (Nathanson et al., 1993; Sasaki et al., 2009; Scheiner et al., 2002).

A second experiment investigated the influence of male density and the presence of females on longevity. Once again, chronic amine treatment was administered. Competition was assumed to serve as a stressor (Zurlini & Robinson, 1980) probably increasing the rate of energy consumption, which should subsequently lead to premature death (Glaser & Kiecolt-Glaser, 2005). Competition was assumed to have the strongest effects on the presence of females. Pursuant to the findings in crickets, amine treatment should increase mortality if the animals are kept in high-density groups and especially in the presence of females. To further unravel longevity-related effects of competition from general sublethal effects of amine treatment, the wasps from the behavioural observations were stored individually up to their death and continuously fed on either diet.

6.3 Methods

6.3.1 Intrasexual conflicts and behaviour

Young male *A. tabida* wasps were collected from their breeding tube within 24 hrs of hatching and kept in isolation in small glass vials filled up to approximately one cm with agar and capped with a foam plug at 20°C and in a 16/8 light/dark cycle until the experiment. The wasps in the experiment were 13-14 days old, as preliminary experiments showed an activity peak around this age.

6.3.1.1 Drug treatment

Dopamine hydrochloride and octopamine hydrochloride were purchased from Sigma-Aldrich, Germany. For both drug treatments, 0.62mg of one of the substances were dissolved in 1 g of honey; pure honey served as control. Honey solutions were placed on the foam plug in *ad lib* amounts and replaced weekly.

6.3.1.2 Experimental set-up

Two males were placed in a 6 cm Petri dish one after the other. The Petri dish was lined with agar and a small patch of baker's yeast measuring 2 cm in diameter located in the centre, with eight early 2nd instar larvae of *D. melanogaster* crawling on it. This patch had been exploited by a female of the same age just prior to the experiment and we expected the olfactory cues to indicate the vicinity of a female. After introduction of the first male, the observation started using The Observer XT 08 (Noldus-Information-Technology, 2008). Scores were awarded for both individuals for the times walking, resting, leg tapping, wing fanning as well as each occurrence and the duration of physical contact of the antenna as agonistic behaviour. Different degrees of escalation were also noted, although not explicitly statistically analysed, as they occurred so rarely.

6.3.1.3 Statistical analysis

All analyses were conducted using the statistical software R.3.2.1 (R-Core-Team, 2014).

Data analysis was conducted using a two-step approach. The first step tested for food-related differences between the observations. Firstly, the total number of physical contacts was analysed. This was started by fitting a generalized linear model (GLM) with quasipoisson distribution and log-link function to the data as count data usually follow a poisson distribution (Hoffmeister et al., 2006) using the *car* library (Fox & Weisberg, 2011). However, this analysis revealed a severe overdispersion (dispersion parameter: 8.286148) and, accordingly, the decision was taken to switch to a zero-inflated poisson model (ZIP) using the *pscl* package (Zeileis et al., 2008) and *lmtest* (Zeileis & Hothorn, 2002) for subsequent model comparison as described in Zuur et al. (2009). Basically, *ZIP* is an adequate solution for counting data with more zeros than expected and solves the problems that arise from this by splitting the model into two parts: one modelling the binomial part (does an event happen at all?) and another one modelling the poisson part (does the number of events differ?). Secondly, the median duration per conflict within each observation was analysed using a GLM with gamma distribution and log-link function as a proxy for intensity (Rillich & Stevenson, 2011; Stevenson et al., 2000) of the physical conflicts. Therefore, all observations without any physical contact were discarded. Lastly, the time needed until the first physical contact occurred was analysed using a survival analysis (Therneau, 2014).

The second part of the analysis was conducted based on the individual wasp rather than on the level of observation. The time was calculated that each individual was engaged in a given behaviour (walking, resting, rubbing the hind legs, or wing fanning) and those times analysed as fractions of the total time spent engaged in the specified behaviours. Analysis was conducted using GEE-GLM (Højsgaard, 2006) with binomial distribution and logit link-function. , “Observation” was specified as the identifier in order to correct for intraobservational dependencies of the data.

6.3.1 Effects of amine -treatment and competition on longevity

6.3.2.1 Survival in experimental animals

All wasps used in the experiment were placed back into their vials after the experiment and were stored at 20°C and 16/8 L/D until their death. Food treatment continued as described above and pieces of agar were added to the vials if and as necessary. Survival was recorded daily. Drug-dependent differences in survival were analysed by means of survival analysis using the *survival* library (Therneau, 2014).

6.3.2.2 Survival in males facing different degrees of intrasexual competition

Animal collection and drug treatment is the same as the method described above with the sole difference being that males were not kept individually but in groups of ten.

6.3.2.2.1 Experimental set up

The experiment used 2-sided, open tubes measuring 2.5 cm in diameter and 6 cm in length. Two of these tubes were taped together, either separated by a thin gauze or open to each other. The double-tubes were capped with foam plugs at both ends. One of the foam plugs was punched through with a pipette tip, which was again covered with gauze. The pipette tip was connected to a rubber tube, which led to an air supplying device emitting a barely noticeable jet of air. An Erlenmeyer flask filled with tap water was placed between the air supplying device and the tube to humidify the streaming air. In addition, two slices of agar were placed at each end of the double tube. The agar was replaced each time it started to dry out.

The ten males were always inserted at the distal end of this apparatus and, if present, the females at the proximal. All in all, four different treatments were prepared for each drug treatment:

1) 10 males, 10 females not separated by gauze (MIX)
2) 10 males, 10 females separated (SEP)
3) 10 males in a separate tube/high density (all-maleHD)
4) 10 males in an open tube/low density (all-maleLD)

Obviously, treatment 1 was the only one to allow mating. In treatment 2, the males can sense the presence of the females but may not access them. In treatments 3 and 4, no females are present at all. Treatment 4 was chosen to elucidate intrasexual and intraspecific competition: While the overall density in treatments 1 to 3 is stable, the density of intrasexual competitors is only half in the first treatment. If males suffer more from the presence of consexual individuals, then they should benefit from the lower density in treatment 1. That may also mask the supposed detrimental effect of mating opportunities.

Survival of the males was noted daily. Dead females were replaced as noticed.

Statistical analysis was conducted by fitting a mixed effects Cox model using the *coxme* library (Therneau, 2012). *Tube* was specified as a random term to correct for tube-specific effects.

6.3 Results

6.4.1 Intrasexual conflicts and behaviour

Competitors came into physical contact in 58.89% of all observations. In many cases, the observed behaviour mirrored that described for fights in crickets (Alexander, 1961). When two competitors encountered each other, they usually stood relatively still, keeping the antennae in contact. Subsequently, the rivals started an intense time of antennal fencing. In most cases, one of the opponents retreated at this point. However, mandible engagement and even grappling was observed in some cases. Particularly after these high levels of escalation, one of the opponents raised its wings and started jerking its body. This is interpreted as a signal of superiority (Alexander, 1961). The defeated animal could usually be clearly distinguished based on a distinct retreat from the opponent. The predicted duration of each contest sequence was with 9.19 s (SEL: 8.06 s, SEU: 10.07 s) a clearly distinct event which was not affected by amine treatment (GLM_{gamma}: $F_{2,35}$= 0.40, p=0.67, see Figure 6.1b).

In the observations in which physical contacts occurred, they occurred frequently (see Figure 6.1a), although significantly less often in OA-treated pairs than in either CONTROL or DA-fed pairs (ZIP: $X^2_{5, 60}$= 13.254, p= 0.001, see Figure 6.1a). While CO- and DA-treated wasps both had around eight physical contacts on average, OA-treated wasps were only involved in a conflict around five times.

Table 6.4: Proportion of time spent engaged in different behaviours split by food as predicted by geeglm analysis with observation as identifier to specify data coming from the same observation. Data were analysed using a binomial data distribution with logit link-function.

	CO (N=49)			DA (N=39)			OA (N=44)			
	SEL	***Mean***	*SEU*	*SEL*	***Mean***	*SEU*	*SEL*	***Mean***	*SEU*	
Walk	0.16	**0.19**	0.21	0.25	**0.28**	0.32	0.20	**0.22**	0.25	df=2, χ^2=5.39, p= 0.07
Wing fanning	0.17	**0.21**	0.25	0.10	**0.12**	0.14	0.13	**0.17**	0.20	df=2, χ^2=4.44, p= 0.11
Resting	0.43	**0.45**	0.48	0.43	**0.46**	0.48	0.43	**0.45**	0.48	df=2, χ^2=0.02, p= 0.99
Leg rubbing	0.14	**0.16**	0.17	0.13	**0.14**	0.15	0.15	**0.17**	0.18	df=2, χ^2=1.64, p= 0.44

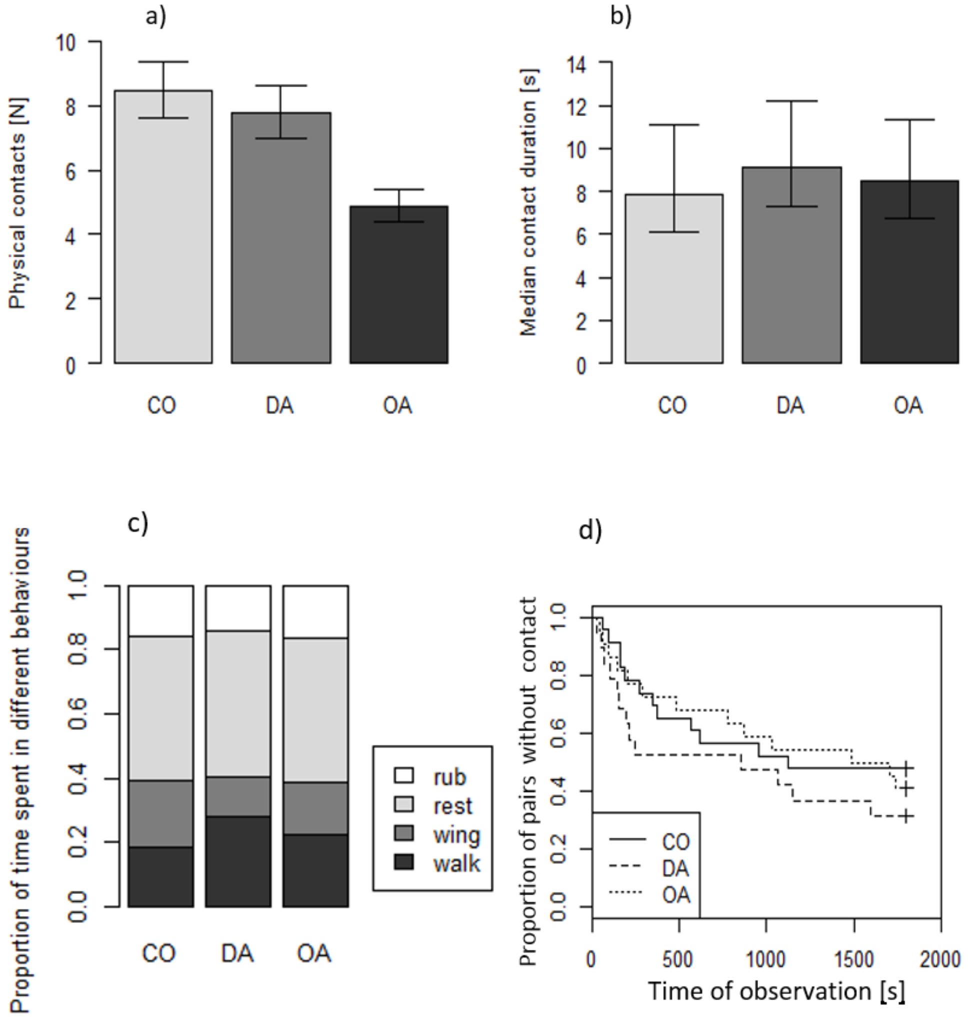

Figure 6.1 Contact behaviours and allocation of time to behavioural categories in Asobara tabida males subjected to different feeding treatments with biogenic amines. a) Number of physical contacts if they occur as predicted by the ZIP-model. b) Duration of physical contacts as predicted by GLM with gamma link-function. c) Proportion of time spent in the different scored behaviours (rub: rubbing the hind legs, rest: standing still, wing: raising or fanning the wings, walk: walking or running through the arena as predicted by GEE-GLM with binomial link-function. d) Kaplan-Meyer survival plot describing the chance of coming into a physical contact during the period of observation as a function of feeding treatment. CO= Control, DA= Dopamine, OA= Octopamine. For further information, please refer to the text.

With 1,123 s (LCL: 372 s, UCL:*, N= 23) in CONTROL, 852 s (LCL: 193 s, UCL:*, N= 19) in DA- and 1,593 s (LCL: 779 s, UCL:*, N= 22) in OA-treated animals, the median latency until the first physical contact was of considerable duration due to the high number of observations without any physical contact. However, the differences between the feeding treatments was not significant (χ^2=1.38, df= 2, p= 0.5, see Figure 6.1d).

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[*] The upper confidence limits could not be calculated due to the high number of observations without any physical contact.

Males spent nearly half of the time standing, while the other half was allocated between walking, rubbing the legs and raising/fanning their wings (see Figure 6.1 c, Table 1). The allocation of behavioural patterns was mostly unaffected by amine treatment, but a strong trend indicates more time spent engaged in walking in DA-treated animals (df=2, χ^2=5.39, p= 0.07, see Figure 6.1 c, Table 6.1), which seems to be at the expense of a slight reduction in wing fanning (see Figure 6.1 c).

6.4.3 Effects of amine treatment and competition on longevity

6.4.3.1 Survival in experimental animals

At 37 days in the CONTROL as well as in the DA treatment and 34 days in the OA treatment, median survival of the experimental wasps did not differ significantly between the different feeding treatments (χ^2= 2.1, df= 2, p= 0.35, see Figure 6.2).

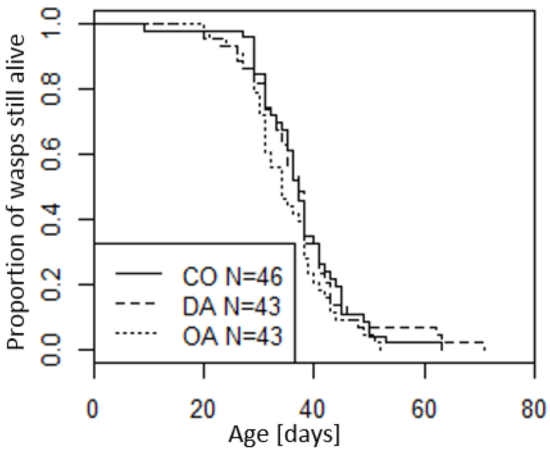

Figure 6.2: Kaplan-Meyer survival curves describing the proportion of male wasps still alive when kept in isolation presented as a function of time and feeding treatment with biogenic amines (CO = control; DA = dopamine; OA = octopamine).

6.4.3.2 Survival in males facing different degrees of intrasexual competition

Survival was best explained by the interaction term of FOOD and KEEPING (χ^2_6= 20.86 df= 6, p= 0.002). OA feeding in general slightly increased the risk of dying (β= 0.69, exp(β)= 1.98, se(β)= 0.33, z= 2.05, p= 0.04) while no general effect of DA was found (β= -0.04, exp(BETA)= 0.97, se(β)= 0.32, z= -0.11, p= 0.93). For a detailed analysis and interpretation of the interaction term, the model was split by feeding treatment. It turned out that survival was highly impacted by the method of KEEPING in all three feeding treatments (CONTROL: χ^2_3= 28.45, p< 0.001; DA: χ^2_3= 35.095, p< 0.001; OA: χ^2_3= 8.695, p= 0.03). However, the effect size was weakest in the OA treatment. Comparing survival between feeding treatments within the different methods of KEEPING reveals some evident differences: In all three feeding treatments, animals

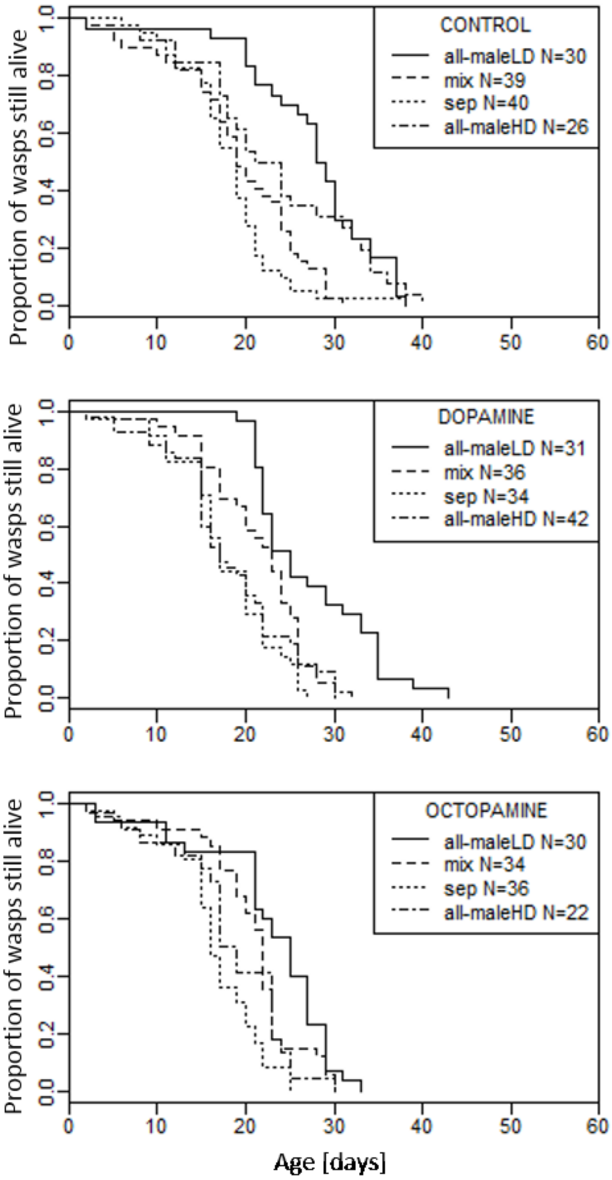

Figure 6.3: Kaplan-Meyer survival curves describing the proportion of male individuals alive at a given age. Separate panels are given for different feeding treatments and capturing methods. From top to bottom: Control, DA, OA with all-male = no females were present, LD = low density, HD = high density, mix = females were freely accessible, sep = females were present, but separated from males.

faced the highest risk of dying if females were present but not accessible (sep, see Figure 6.3) and lived longest if they were kept in the absence of females in low densities (all-maleLD, see Figure 6.3). Comparing the impact of the different methods of KEEPING against the all-maleLD group reveals that while in the CONTROL group high male density (all-maleHD) had no additional effect on survival (β= 0.25, exp(β)= 1.29, se(β)= 0.27, z= 0.93, p= 0.35), it strongly increased the risk of mortality risk in DA-treated animals (β= 1.80, exp(β)= 6.08, se(β)= 0.32, z= 5.59, p< 0.0001) and had an intermediate and nearly significant effect in OA-fed males (β= 0.71, exp(β)= 2.04, se(β)= 0.38, z= 1.89, p= 0.06, see Figure 6.3). On the other hand, access to females (mix) only increased the risk of mortality in the control group (β= 1.01, exp(β)= 2.75, se(β)= 0.25, z= 3.97, p< 0.0001) and the DA-fed group (β =1.14, exp(β)= 3.12, se(β)= 0.45, z= 2.51, p= 0.01), but not in OA-treated wasps (β= 0.43, exp(β)= 1.53, se(β)= 0.28, z= 1.54, p= 0.12).

6.5 Discussion

This study aimed to investigate competitive behaviour in males of *Asobara tabida* which had been isolated from conspecifics for a considerable time. Furthermore, it tested the influence of oral DA and OA administration respectively, as both substances have been shown to be important agents in aggressive behaviour in other arthropods such as crickets (Rillich et al., 2011; Rillich & Stevenson, 2011; Stevenson et al., 2000), fruit flies (Certel et al., 2007; Dierick, 2008; Hoyer et al., 2008; Zhou et al., 2008) and crustaceans (Antonsen & Paul, 1997; Benelli et al., 2013; Joyce et al., 2014). Finally, the effects of different degrees of intrasexual competition and amine treatment on longevity were analysed as measurements for sublethal effects of amine treatment and as an indicator of consexual-induced stress (Adamo & Baker, 2011; Zurlini & Robinson, 1980).

Interestingly, relatively little is known about intrasexual competition in males parasitic wasps (but see Godfray, 1994; van den Assem et al., 1980), with this being especially true for solitary, pseudogregarious species. Still, conditions faced in pseudogregarious species should clearly favour competition between males as, as far as it is known, sex ratios are usually relatively balanced and good quality females become a limited resource for males (Carton et al., 1986; Fauvergue et al., 1999). To the authors' knowledge, a detailed description of male aggressive behaviour in *A. tabida* has never been published and this work served as a kind of pre-test investigating whether aggressive encounters occur at all. Therefore, it was decided to isolate the animals prior to the experiments as isolation from conspecifics has been shown to enhance aggressiveness (Benelli et al., 2012; Stevenson & Rillich, 2013) and, furthermore, the subjective value of cues derived from a female should increase as well if access to females is rare (Godfray, 1994). It was found that intra-male agonistic behaviour occurs frequently under the aforementioned circumstances and, what's more, it mirrors in its levels of escalation those described in crickets in many respects (Alexander, 1961). Although the level of escalation does not exceed antennal fencing on most occasions, mandible engagement and grappling also occurred. The winner signalled its superiority by raising up its wings and jerking its body. Interestingly, if agonistic encounters occurred, they were rather frequent, but in more than 40% of all observations they never occurred at all. The next suggested step would be to investigate which circumstances promote the likelihood that the animals will engage in fights. For example, it is possible that contestants were able to recognise the competitor's qualities in advance from a distance. If the outcome of a potential contest can be reliably predicted, it may make sense for the inferior individual simply to avoid it rather than accepting the costs of fighting and losing (Arnott & Elwood, 2008; 2009).

When not engaged in a contest, males remained standing for nearly half of the observational time, with the rest of the time spent simply walking or running through the arena, fanning or keeping up their wings or rubbing their hind legs. In the context of mating, wing fanning is the most conspicuous sign of courtship behaviour (Dufour et al., 2012; Villagra et al., 2011) and it has been hypothesized that the proximate function of wing fanning in courtship is to produce an airstream transporting the female's volatiles in order to facilitate orientation towards the female (Villagra et al., 2011). In our experiment, the arena was previously visited by a female, likely depositing some odour cues during her visit (Dufour et al., 2012; Stoekl et al., 2014). Accordingly, wing fanning might simply be attributable to the former presence of the female and be merely a sign of its recognition. However, as wing fanning occurred mostly when males were standing face-to-face and was also used by the winning male to signal its superiority, it probably plays a distinct role in intrasexual communication as well. Approximately the same amount of time the animals spent fanning their wings was spent rubbing their hind legs. It is likely that this behaviour produces some stridulatory sounds. Stridulation in intrasexual communication has rarely been investigated in groups other than crickets, (but see (but see Boon et al., 2009; Schmidt, 1994)). Nevertheless, it has been argued that stridulatory patterns may provide contestants with information about the competitor's physical fitness (Schmidt, 1994) at minimal energetic costs (Hack, 1997). Taken together, male agonistic behaviour shows a strongly ritualized repertoire, from which one can conclude that male-male competition is a serious challenge in this species even under natural conditions. It should be noted that it has been observed that male-male competition seems to decrease in intensity with time of rearing in the lab in a number of parasitoid species (van den Assem et al., 1980). Against that background, it may be carefully hypothesized that under natural conditions even more agonistic encounters would occur and escalate.

Amine treatment affected agonistic behaviour in the opposite way than was expected. DA treatment had no distinct effect on the number of agonistic encounters while OA treatment actually decreased it, although both substances were in fact expected to increase the likelihood of fights, as blocking of either substance was shown to have the opposite effect in numerous studies on crickets (Rillich et al., 2011; Rillich & Stevenson, 2011; Stevenson et al., 2000) and fruit flies (Certel et al., 2007; Dierick, 2008; Hoyer et al., 2008; Zhou et al., 2008). This said, the aforementioned studies all worked with OA-depleted animals, using pharmacological approaches in the case of the cricket studies and mutants in the case of the *Drosophila* experiments. Perhaps it is an oversimplification to assume a somehow linear relationship of intrinsic OA concentrations and aggressiveness. Although the majority of studies testing the effect of OA injections deal with crustaceans (Antonsen & Paul, 1997; Momohara et al., 2013), they consistently show a decreased likelihood to fight, as does O-depletion in the above-mentioned studies. Unfortunately, the corresponding experiments testing the effect of OA application in flies and crickets and amine depletion in crustaceans are missing, so it cannot be ruled out that the results are group specific. However, as the aminergic system seems to be highly conserved within the arthropods in general (Verlinden et al., 2010), this seems to be unlikely, especially as the authors' results on hymenopteran parasitoids now head in the direction of the ones revealed from studies on crustaceans. Contrarily, it may be the case that the relation of OA concentration and aggressive behaviour is bell-shaped rather than linear as it is supposed for the norepinephrinic system, the OA's counterpart in vertebrates (Aston-Jones & Cohen, 2005). However this still needs to be tested.

DA treatment had no visible effect on either the number or the duration of agonistic encounters. This may be due to rapid oxidation of orally administered DA prior to its absorption (Scheiner et al., 2002). However, in other studies, oral DA treatment was shown to enhance DA titres in the brain significantly (Sasaki et al., 2009) and affect behaviour (Agarwal et al., 2011), and, what's more, the authors found a strong tendency for an increased amount of time spent walking in DA-treated animals. Even though the opposite has been reported (Mustard et al., 2010), this seems to be a typical effect evoked by DA treatment (reviewed in Yamamoto & Seto, 2014) and can be taken as an indication for successful uptake of DA.

Neither amine treatment caused an effect on longevity in wasps kept in isolation, but the animals' diet strongly affected longevity if the males were kept in groups. As such, one can exclude the notion that amine-treatment per se has any long-term sublethal effect in general. Irrespective of the feeding treatment, it was beneficial for all animals to be kept at low densities. However, the reduction in lifespan upon increasing the number of individuals was not simply the result of higher energetic costs, e.g., caused by a higher chance to stumble over one another and forcing each other to give way (Zurlini & Robinson, 1980), as distinct differences between the approaches with high numbers of individuals per unit space exist. In all three feeding treatments, the risk of mortality was highest if male density was high and females were present but not accessible (sep). This was only to be expected, as the perceivable vicinity of the females should evoke intense courtship behaviour as well as a high level of intrasexual competition, both increasing the demands on energy (Ahtiainen et al., 2005; Hack, 1997; Watson et al., 1998). Furthermore, the results indicate that intrasexual competition is a more severe stressor than intersexual competition as males in the mix treatment lived considerably longer, even though overall animal density was the same and females were also present. Nevertheless, the fact needs to be considered that mating was possible in the sexually mixed approach, which alters the status of the female. Although males of *A. tabida* even display courtship behaviour even towards mated females, they do so more intensively if the female was mated previously by another male and the intensity of courtship towards mated females is lower overall compared to virgins (Louapre et al., 2014). As such, to clarify finally if the more pronounced reduction in lifespan in separated males was due to higher costs of intrasexual competition compared with intersexual competition, a set-up needs to be developed in which mating is allowed but the status of the females remains constant, probably by replacing all females regularly.

The significant interaction of feeding treatment and the way of keeping the wasps was mainly caused by two effects. Firstly, OA-treated animals faced a generally higher tendency to die and, secondly, the risk of mortality in the wasps kept without females but at a high density (all-maleHD) was considerably higher than in the control group in both amine treatments. The first effect is in line with others focusing on the role of OA on the stress response (Adamo & Baker, 2011). The authors found a number of stress-related physiological responses to correlate with increased OA titres and further OA application has been shown to mimic the occurrence of predator-caused stress (Adamo et al., 2013) and increase metabolic rate (Arrese & Soulages, 2010; Roeder, 2005). The latter has been shown to be crucial for longevity, especially in parasitoids as they are unable to restore their lipid reserves (Seyahooei et al., 2011). However, it does make one wonder that this effect is not found in the wasps kept in isolation. This may indicate that the combination of somehow stressful stimuli and OA treatment is required to unfold the effects on longevity caused by OA.

The second effect indicates that both amines do indeed play a role in intrasexual behaviour. It should be expected that in the absence of females to compete for, intrasexual competition should only play a minor role, as is found in the control group, whereas the all-maleHD group had the second longest life expectancy. However, in both amine treatments, mortality corresponded to the data found for males with females present rather than the ones for the all-maleLD treatment. This indicates that both amines increase the stress level induced by the presence of conspecific males.

This work shows that the application of OA and DA affects behaviour, which in turn impacts longevity in male parasitic wasps. It is noticeable that the effects of amine treatment are more prominent in males than in females (see Chapter 5) but there are still many aspects which need to be unravelled: Is the decreased likelihood to fight in OA-treated animals caused by an increased stress level making both contestants prefer to avoid further conflict? Is the density-dependent impact of DA treatment on longevity a consequence of its effect on walking activity as the more an animal walks around the higher the likelihood to encounter a competitor? Does OA-induced stress increase energy expenditure and can this be quantified? Furthermore, does this effect differ depending on the presence/absence of additional stressors? This list is by no means exhaustive. However, although this work probably poses more questions than can be answered, it brings into the field the usefulness of male parasitic wasps for the study of behaviour and the role of biogenic amines therein. The fact that the responses of males and females are so distinctively different in these animals may make this system particularly useful for the study of the physiological basis of behavioural responses.

6.6 References

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7 General discussion and conclusion

The aim of this work was to investigate proximate mechanisms underlying adaptive host searching and patch-leaving behaviour in parasitoid wasps. The necessity of merging the findings with respect to ultimately optimal behaviour and the physiological mechanisms involved has recently been claimed repeatedly (Hoedjes et al., 2010; McNamara & Houston, 2009; Nathan et al., 2008; Pierre, 2011). Optimal foraging theory assumes that every unnecessary cost invested is a setback in terms of the ultimate goal of fitness optimization and a high selective pressure should bear on the evolution of economically optimal searching strategies (Stephens & Krebs, 1986). Thus, the same assumption should hold true for the physiological costs involved in adaptive searching behaviour. For example, an observed behavioural strategy may be suboptimal at first glance, but becomes the most economic strategy when physiological costs like information processing and memory storage are taken into account. The general underlying assumption in all of the studies presented here is that parasitoids experience different events during their search that they perceive as either rewarding (i.e., host encounter, host cue perception) or negative (i.e., predator cues, competitors, patch borders, etc.). These pieces of information serve for the animal as cues taken to adapt behaviour. The value of these different cues is not fixed, but depends on the physiological state and experience of the animal. The main question arising from this assumption was which heuristic, low-cost mechanisms enable state-dependent adaptive responses to these different cues.

The first study tested a heuristic movement model (Wajnberg et al., 2013) in which different events elicit certain locomotory responses in the animal, either intensifying foraging behaviour or weakening it. Patch leaving would be a consequence of a gradual waning searching intensity leading to a straighter walking path and higher velocity over time, whereby different events elicit certain locomotory responses, expressed either as a reintensification of or a further decrease in searching intensity. This and other movement models (Benhamou, 2007; Fronhofer et al., 2013) are inspired by field observations on different species indicating such a movement pattern. However, it was unclear whether such a movement pattern occurs due to a proximate searching mechanism or if it is a reflection of gradual patch depletion caused by exploitation activity logically leading to larger intra-prey distances (Bazazi et al., 2012). The results showed only weak correlations of the locomotory responses and the model assumptions in both of the studied species, indicating that ultimate rather than proximate causes lead to the observed walking phenomena in field studies. What’s more, the pattern found fitted well into the framework of reward and reward prediction, as it is nicely explained for vertebrates (Schultz et al., 1997; Wise, 2004). Basically, neuronal activity in vertebrates responds to cues which promise rewards rather than to the reward itself if the experimental animals are well experienced with the set-up (Wise, 2004). Additionally, if a specific reward fails to appear within circumstances usually resulting in the achievement of a reward, the animals respond clearly negatively. A similar reaction has been found before in *L. heterotoma* and the animals avoid formerly preferred sites if they experience a strong mismatch of hosts actually present and their (learned) expectations regarding the patch quality (Papaj et al., 1994). In line in Chapter 2, parasitoids of *L. heterotoma* show a noticeably stronger locomotory response towards the host-derived cues than towards the encounter of the host itself, whereas *V. canescens* displayed stronger responses to the host encounter. Considering the predictive value of host-derived cues with respect to the presence and number of available hosts in both species, this type of pattern is actually to be expected. This is because the thelytokous strain of *V. canescens* used here is adapted to surroundings with a strong human impact in which the number of hosts is highly variable due to human pesticidal actions (Skovgard et al., 1999), while the presence and concentration of host-derived cues serve as a reliable predictor for the presence and number of hosts in *L. heterotoma*.

The main neurological pathway involved in the framework of reward and reward prediction mentioned above (Schultz et al., 1997; Wise, 2004) is the dopaminergic system. Due to its role in reward perception and modulation dopamine is commonly known to act as a kind of “happiness hormone”. However, in invertebrates, the function of dopamine seems to be an essentially different one: rather than modulating the response to rewards, it seems to be crucial in the assessment of aversive stimuli (Agarwal et al., 2011; Mizunami & Matsumoto, 2010; Mizunami et al., 2009; Schwaerzel et al., 2003; Unoki et al., 2005; Unoki et al., 2006), whereas octopamine seems to work as a kind of invertebrate analogon to dopamine in the reward system (Barron et al., 2007; Hammer & Menzel, 1998; Menzel et al., 1999; Mizunami &

Matsumoto, 2010; Mizunami et al., 2009; Schwaerzel et al., 2003; Unoki et al., 2005; Unoki et al., 2006).

Based on the above-mentioned main assumption that the behaviour of parasitoids should depend on the assessment of different, positive and negative, events and the indications from the first study, all the rest of the studies presented in this thesis involved the manipulation of the intrinsic octopamine and dopamine concentration respectively, with a slightly stronger focus on the role of octopamine. All but the last experimental set-ups contained rewards in terms of hosts and different potential costs. The costs included simple costs like time and energy (Chapter 3), a potential risk of predation (Chapter 4) and the costs arising from direct and indirect competition (Chapter 5 and 6). It was assumed that the impact of octopamine and dopamine on the subjective reward value could be estimated from the costs invested (Arnott & Elwood, 2008).

7.1 Effects of dopamine on host searching and patch-leaving behaviour

The effects of the oral administration of dopamine were tested in Chapters 4, 5 and 6. Basically it was assumed that dopamine-treated animals should be less willing to continue searching if confronted with potentially negative stimuli in forms of a mimicked predator (Chapter 4) or competitors (Chapter 5) and to show a decreased likelihood to fight for potential rewards (Chapters 5 and 6). This assumption was based on numerous studies showing that pharmacologically dopamine-depleted animals (Agarwal et al., 2011; Mizunami et al., 2009; Unoki et al., 2005; Unoki et al., 2006; Vergoz et al., 2007) or genetic mutants (Schwaerzel et al., 2003) fail to learn aversive stimuli. For example, while untreated animals learn to avoid sites in which an electric shock (Agarwal et al., 2011; Schwaerzel et al., 2003; Vergoz et al., 2007) or saline water sources (Unoki et al., 2006) can be expected, dopamine-depleted animals continue to visit such sites. Another study into intra-male competitive behaviour hypothesizes the same mechanism to be responsible for an ongoing high tendency to fight in dopamine-blocked inferior crickets (Rillich & Stevenson, 2014). The authors conclude that these animals fail to assess the aversive experience of being defeated. Still, the results of the studies presented here did not show any effects of dopamine treatment on the assessment of aversive stimuli. Other studies also reveal ambiguous results with respect to behavioural modulations induced by oral dopamine treatment and different reasons have been suggested. Firstly, it has been hypothesised that the chemically relatively instable dopamine oxidises quickly in the intestine and that actually only marginal amounts of dopamine cross from the intestine to the haemolymph (Scheiner et al., 2002). Furthermore, it may be that dopamine works in more specific regions compared to the broadband octopamine (Menzel et al., 1999) and simply does not reach the specific regions responsible for the assessment of aversive events via an unspecific application. Lastly, it is possible that the relative additive effect of the administered dopamine was too small to evoke significant responses, as the natural concentration in the honeybee brain at least is 2-3 times that of octopamine (Harris & Woodring, 1992; Roeder, 1994). Nevertheless, in *Drosophila*, it is the other way around (Hardie & Hirsh, 2006). However, other studies revealed significant effects of oral dopamine treatment (Agarwal et al., 2011; Sasaki et al., 2009). In the study conducted by Agarwal et al. (2011), feeding dopamine led to a significantly stronger avoidance response to aversive stimuli even within the behavioural training and not only in a subsequent memory test. This is similar to what was tested in the studies presented here, as, basically, a reduced response threshold towards aversive stimuli was expected with oral dopamine administration. Although this was not retrieved, the results in Chapter 6 indicate that at least some dopamine crosses the gut wall, as dopamine-treated animals showed a strong tendency for an altered locomotory pattern expressed in more time spent walking compared to the control and the octopamine groups. An enhancing effect of dopamine on walking behaviour is a common effect in invertebrates (reviewed in Yamamoto & Seto, 2014). Thus, the next suggested step would be to test which amounts of dopamine actually reach the wasps metabolism and in which regions it can be retrieved.

7.2 Effects of octopamine on host searching and patch leaving behaviour

How the oral administration of octopamine affects host searching and patch-leaving behaviour in parasitoid wasps was investigated in Chapters 3, 4, 5 and 6. Based on numerous findings mostly in drosophilids (Scheiner et al., 2014; Schwaerzel et al., 2003), bees (Barron et al., 2007; Barron et al., 2002; Behrends & Scheiner, 2012; Erber & Kloppenburg, 1995; Hammer & Menzel, 1998; Scheiner et al., 2006; Scheiner et al., 2002) and crickets (Mizunami & Matsumoto, 2010; Mizunami et al., 2009; Nakatani et al., 2009; Unoki et al., 2005; Unoki et al., 2006) showing the modulatory role of octopamine on setting response thresholds (Barron et al., 2007; Barron et al., 2002; Behrends & Scheiner, 2012; Pankiw & Page, 2003; Scheiner et al., 2014) and learning

(Hammer & Menzel, 1998; Mizunami & Matsumoto, 2010; Nakatani et al., 2009; Unoki et al., 2005; Unoki et al., 2006) of appetitive stimuli, it was assumed that octopamine-treated animals should exploit a patch with a given number of hosts more intensively (Chapter 3), to show a higher likelihood of remaining in the face of a severe risk (Chapter 4) and defending potential resources against competitors more intensively (Chapters 5 and 6). However, the revealed effects of octopamine do not serve to support these hypotheses, although distinct behavioural modulations were indeed found. Firstly, it should be mentioned that while earlier results for oral dopamine treatment are ambiguous (see above), oral octopamine treatment seems to be a generally efficient and minimally invasive approach (Agarwal et al., 2011; Barron et al., 2007; Barron & Robinson, 2005; Barron et al., 2002; Liang et al., 2012; Scheiner et al., 2002) and significantly affects behaviour across species. However, it has been criticised that the effects with respect to reward modulation in particular are predominantly derived from nutritional – or more specifically sucrose – rewards (Perry & Barron, 2013). To account for this, it was assumed that bringing rewards in forms of oviposition opportunities into the field would be a good approach for testing the generality of the modulatory role of octopamine in reward assessment. Other studies using non-sucrose rewards revealed conflicting results. Whereas in some studies similar results are found to those in set-ups containing sucrose (Mizunami & Matsumoto, 2010; Nakatani et al., 2009; Unoki et al., 2006), others fail to identify a comparable octopamine effect (Erber & Kloppenburg, 1995; Sitaraman et al., 2010). However, the numerous studies on aggressive behaviour in octopamine depleted crickets in particular (Rillich et al., 2011; Rillich & Stevenson, 2011; Rillich & Stevenson, 2015; Stevenson et al., 2005; Stevenson et al., 2000; Stevenson & Rillich, 2012) and octopamine-knockout *Drosophila* mutants (Baier et al., 2002; Dierick, 2008; Hoyer et al., 2008; Zhou et al., 2008) seem to promote the generality of the reward hypothesis. The mentioned studies on crickets unanimously conclude that octopamine-blocked animals do not estimate the reward value of a preferred shelter site as highly as control animals do, which should explain the decreased likelihood to fight. However, other authors suggest that it is not the role of octopamine in reward assessment which leads to the observed decreased aggressiveness in octopamine-depleted animals, but rather its role in energy metabolism (Dierick, 2008). Octopamine has long been known to be essential for the activation of energy reserves and fat metabolism (Roeder, 1999; 2005). In the face of energy-demanding circumstances (like fight, flight, stress), octopamine concentrations in the haemolymph usually increase and fat reserves are activated (Roeder, 2005). Accordingly, the observed decreased aggressiveness in octopamine-depleted animals may simply be the result of an inability to fight due to a lack of available energy resources, rather than being related to an effect in reward assessment (Dierick, 2008). Against this background, the strong effects of octopamine treatment within nutritional reward set-ups become rather clear. If the metabolic rate correlates with octopamine concentrations, then animals with an artificially raised octopamine titre have higher metabolic costs and a higher need to refill their energy demands and vice versa. In line with an increased energy expenditure in the cause of octopamine treatment, the authors only recently found indications for a higher risk of mortality in octopamine treated parasitoids of *V. canescens* suffering food deprivation compared to untreated wasps (Gerdts, 2015).

As mentioned above, increased energetic demands usually occur in stressful situations. Correspondingly, octopamine has been shown to be a central modulator in invertebrate stress response (Adamo & Baker, 2011; Adamo et al., 2013; Harris & Woodring, 1992; Roeder, 1999; 2005). In total, the results achieved within this thesis support this role. Females of *L. heterotoma* needed longer to enter the patch, displaying at the same time higher exploitation efficiency (see Chapter 3) consistent with findings on human task performance under stress (Starcke & Brand, 2012) and with other findings concerning the searching behaviour of *L. heterotoma* under physiological stress (Rafalimanana et al., 2002). Furthermore, they were more likely overall to leave the patch upon perception of a danger cue (Chapter 3), similar to what has been found in octopamine-treated crickets (Adamo et al., 2013). The fact that octopamine-treated animals appeared not to be able to adapt their behaviour to the rate of exploitation can be taken as a typical sign of decision-making under stressful conditions again (Starcke & Brand, 2012), as the experimental subject probably does not scan all the alternatives properly before deciding. Finally, male pairs fed octopamine were involved in physical contests less often than untreated or dopamine-fed individuals (Chapter 6). As mentioned above, this finding is completely contradictory with respect to the argument based on the results from studies into octopamine-depleted crickets (Rillich et al., 2011; Rillich & Stevenson, 2011; Rillich & Stevenson, 2015; Stevenson et al., 2005; Stevenson et al., 2000). However, evidence revealed by octopamine measurements (Sneddon et al., 2000) and injections

(Antonsen & Paul, 1997; Momohara et al., 2013) in several crustaceans shows that this seems to be a common effect. These studies were able to show that octopamine concentrations increase as a result of losing (Sneddon et al., 2000), that octopamine-treated animals have a higher chance of losing fights (Momohara et al., 2013) and that they display more submissive behaviour overall (Antonsen & Paul, 1997; Sneddon et al., 2000). Considering these results, it becomes reasonable that octopamine-treated males of *A. tabida* avoid further conflict in the experiments presented here. Nevertheless, whether it is indeed the modulatory role of octopamine in the stress response which leads to an avoidance of physical contests, as being inferior and facing a competitor is probably quite stressful, remains open to interpretation and requires further investigation. In fact, throughout the vertebrate family, increased levels of stress hormones have been shown to be characteristic of winners and losers depending on the species, whereby specific effects in invertebrates are still largely unknown (Briffa & Sneddon, 2007).

7.3 Concluding remarks concerning the pharmacological approach

This thesis aimed to test heuristic mechanisms enabling quick behavioural adaptations in parasitoid searching behaviour. A focus was put on the assessment of rewards and costs. Based on evidence from other species, the prominent neuromodulators octopamine and dopamine were assumed to be central agents in this process. However, the results did not meet the expectations. This may have a number of different reasons. Firstly, it should be considered that basically two main approaches exist for testing the effects of neuromodulators: They are either pharmacologically or genetically blocked or administered above the natural level, as was the case throughout this thesis. Both approaches have benefits and disadvantages. A complete knockout of a specific system may cause numerous subtle side effects, whereas most pharmacological receptor antagonists used for reversible blocking of a given neuromodulator do not act completely specifically on the target receptor but also on others (Roeder, 2005). On the other hand, enhancing the levels of a given substance above the normal (likely optimal) threshold may also have a detrimental effect (Menzel et al., 1999). Consequently, conclusions with respect to the natural role of any substance need to be drawn with caution. Furthermore, it has been pointed out above that similar effects of the additional application of octopamine and, conversely, its blockade have been found, which may indicate that bell-shaped rather than linear relationships in the effects of a given substance exist, as they seem to be common in neurotransmitters (Aston-Jones & Cohen, 2005; van Swinderen & Andretic, 2011). To consider this, studies need to be conducted which combine both approaches, blocking and additional application, in a single set-up. Secondly, the method of application used may cause different effects. Dopamine as well as octopamine serve as neurotransmitters as well as neurohormomes and neuromodulators in both the central nervous system and the peripheral organs (Farooqui, 2007). Depending on their function within the animal's physiology and its location, they may cause different effects (Farooqui, 2007; Roeder, 1999; 2005). Although increased brain titres of both substances due to oral administration have been found in other species (Sasaki et al., 2009; Schulz & Robinson, 2001) it remains unclear where in the animal's metabolism this method causes its strongest effects. Unravelling the effects of amine treatment within peripheral organs and the central nervous system requires precise methods of application. However, in parasitoids at least, the problem of precisely administering substances remains challenging as brain injections are hardly possible due to their minute size and thick head capsule, while an injection into the abdomen is rather unspecific, as is oral administration. One possible solution to this problem might be the use of HPLC, for example to get further insights into the pathways taken by orally administered amines within the parasitoids' metabolism.

7.4 References

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Declaration/Erklärung

Gemäß §6 (5) der Promotionsordnung der Universität Bremen für die mathematischen, natur- und ingenieurwissenschaftlichen Fachbereiche, erkläre ich hiermit, dass ich die vorliegende Arbeit mit dem Titel

Experimental approaches to unravel proximate mechanisms of parasitoid searching and patch leaving behaviour

1. selbständig verfasst und geschrieben habe,
2. außer den angegebenen Quellen und Hilfsmitteln keine weiteren verwendet habe, und
3. die den benutzten Werken inhaltlich oder schriftlich entnommenen Stellen als solche kenntlich gemacht habe.

Ebenfalls erkläre ich hiermit, dass es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

Bremen,

Jennifer Uhlig-Herrmann

Lebenslauf

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Beruflicher Werdegang

09/2010 – heute **wissenschaftliche Mitarbeiterin im FB2 der Uni Bremen**

in unterschiedlichen Projekten z.B.:

- 2-jähriges Projekt zur Entwicklung von Lerntagebüchern und Kompetenzportfolios in der Lehre im Rahmen der MINT-Förderung zur Neugestaltung der Studieneingangsphase
- Erstellung einer Online-Lerneinheit
- Elternzeitvertretung zur Aufrechterhaltung der Lehre

07/2007 – 08/2010 **Honorarkraft im Bereich E-Learning an der Uni Bremen**

im Rahmen der Elternzeit und zur Überbrückung

Beispielprojekte:

- Entwicklung eines Online-Seminars zu einer öffentlichen Vorlesungsreihe
- Entwicklung von kursbegleitenden Online-Quizzes.
- Entwicklung von veranstaltungsbegleitenden Lernmodulen

Ausbildung

10/2001 – 06/2007 **Studium der Biologie in Köln und Bremen**

mit Diplom (Abschlussnote: sehr gut)

Studienschwerpunkte: Ökologie, Zoologie

03/2001 – 10/2001	**Studium der Sozialwissenschaften in Göttingen** (ohne Abschluss)
06/2000	**Abitur** Am Alten Gymnasium in Bremen

Auslandsaufenthalte

09/2000 – 12/2000	**Als Volontärin in Botswana, Afrika** in einem Jugendzentrum und in einem AIDS-Waisenprojekt

Publikationen

b) Lehre

Uhlig, J., Hoffmeister, TS. (2015): Workshop on Natural Sciences. In: Schelhowe, H., Schaumburg, M., Jasper, J. (eds), Teaching is Touching the Future. Academic Teaching within and across disciplines (p. 165-168). UniversitätsVerlagWebler, Bielefeld

Uhlig, J., Hansen, E., Hoffmeister, TS.: Wissen oder Können? ePortfolios zur Stärkung des kompetenzorientierten Lernens im BSc. Biologie. Resonanz – Magazin für Lehre und Studium an der Universität Bremen, (http://blogs.uni-bremen.de/resonanz/2013/10/07/eportfolios/)

Uhlig, J., Thiel, A., Hoffmeister TS. (2105): Studierendenzentrierte Betreuung mit E-Portfolio – Ein Erfahrungsbericht aus der Biologie. Resonanz – Magazin für Lehre und Studium an der Universität Bremen, (https://blogs.uni-bremen.de/resonanz/2015/04/20/e-portfolio-biologie/)

Konferenzen

a) Forschung

19. Graduiertentreffen der Fachgruppe Evolutionsbiologie der DZG, Bremen 2014

43rd Annual Meeting of the Ecological Society of Germany, Austria and Switzerland (GFÖ), Potsdam 2013

104. Annual meeting of the German Zoological Society (DZG), Saarbrücken 2011

Entomology Congress of the DGaaE, Berlin 2011

b) Lehre

Final Conference of the European Learning Network (eLene2learn), Barcelona, 2014

Teaching is Touching the Future – Academic teaching within and across disciplines, Bremen 2014 (Co-Chair)

Wissen und Können: Kompetenzziele, Lernergebnisse und Prüfungen studierendenzentriert formulieren und gestalten – Zwischenbilanztagung des HRK-Projektes Nexus, Köln 2013

Jubiläumstagung: 10 Jahre e-teaching.org, Tübingen 2013

8. Stud.IP-Tagung, Göttingen 2010

Vorträge und Posterpräsentationen

a) Forschung

Uhlig, J.[1]*, Thiel, A., Hoffmeister, TS.: The role of biogenic amines on parasitoid´s foraging behavior. 19th DZG evolutionary biology graduate meeting, Bremen 2014 (Vortrag)

Crummenerl, L.*, **Uhlig, J**., Hoffmeister, TS.: The combined influence of a danger cue and oviposition experience on the patch leaving response of parasitoid species with different patch leaving rules. 19th DZG evolutionary biology graduate meeting, Bremen 2014 (Vortrag)

Hoffmeister, TS.*, Wajnberg, E., Coquillard, P., Thiel, A., **Uhlig, J**.: What makes a forager leave a resource patch? Confronting models with reality. ASAB Winter Conference, London 2013 (Poster)

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[*] Präsentierender Autor

Uhlig, J.*, Thiel, A., Hoffmeister, TS.: The role of the neuromodulator octopamine on foraging behavior in parasitoids 43rd Annual Meeting of the Ecological Society of Germany, Austria and Switzerland (GFÖ), Potsdam 2013 (Vortrag)

Uhlig, J.*, Hansen, E., Thiel, A., Hoffmeister, TS.: Neurotransmitter dependent plasticity in reward assessment. 104. Annual meeting of the German Zoological Society, Saarbrücken 2011 (Poster)

Uhlig,J.*, Thiel, A., Hoffmeister, TS: Octopamin- und dopminabhängige Neuromodulation des Suchverhaltens bei Parasitoiden. Entomology Congress of the DGaaE, Berlin 2011 (Poster*)*

Hoffmeister, TS.*, **Uhlig, J.** *et al*.: Does the searching pattern of foragers reveal their motivational status? Entomology Congress of the DGaaE, Göttingen 2009 (Vortrag*)*

b) Lehre

Thiel, A.*[2], **Uhlig, J.***, Bernhardt, T.*: Student engagement with ePortfolios and peer tutoring. eLene2learn Final Conference, Barcelona 2014 (Vortrag)

Uhlig, J.*, Thiel, A., Hoffmeister, TS., Student´s Office Biology/Chemistry, ZMML: ePortfolios/Learning Diaries as a Tool to Improve Self-Learning Capacities. Transnational Workshop on Blog and E-portfolio, 2013 (Online-Vortrag)

Uhlig, J.*, Salm, M., Hansen, E., Hoffmeister, TS.: Kompetenzen im Blick: Studienbegleitende ePortfolios im BSc Biologie. Zwischenbilanztagung des HRK-Projektes Nexus, Köln 2013 (Poster)

Uhlig, J.*: Kompetenzen im Blick: Studienbegleitende ePortfolios im BSc Biologie. Jubiläumstagung 2013: 10 Jahre e-teaching.org, Tübingen (Vortrag)**

Uhlig, J.*, Thiel, A., Hoffmeister, TS.: Stud.IP – die späte Einsicht in die didaktische Neuerung oder die Notwendigkeit des E-Learning in Zeiten knapper Kasse. 8. Stud.IP-Tagung, Göttingen 2010 (Vortrag)

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**dieses Projekt wurde auf eTeaching.org als “best practice” Beispiel ausgezeichnet

Uhlig, J.*, Hoffmeister, TS., *et al.*: Kompetenzen im Blick - Studienbegleitende (Kompetenz-) Portfolios im B.Sc. Biologie. Dies Academicus, Bremen 2013 (Vortrag)

Fördermittel

Fördermittel zur Neugestaltung der Studieneingangsphase in den Naturwissenschaften, der Informatik und den Ingenieurswissenschaften, Universität Bremen 2011: Mitautor des Antrages: Studienbegleitende Kompetenzportfolio als (A) studienverbessernde Maßnahme und (B) QM-Maßnahme (Förderung für eine halbe Stelle von 2012-2014)

Fördermittel im Rahmen des DAAD-RISE Programms zur Förderung des Studierendenaustausches von Nordamerika und Großbritannien nach Deutschland für ein 9-wöchiges Stipendium an Cynthia Chai, 2012

Fördermittel im Rahmen des Programmes „Win a tutor – Didaktische Anwendungsszeanrien mit Stud.IP“ des ZMML, Universität Bremen, (Förderung der genannten Projekte für je ein Semester)

- Restrukturierung eine ILIAS-Quizzes und Implementierung von PINGO zur veranstaltungsbegleitenden Evaluierung der Lernergebnisse: Mitautor 2014
- Einführung der E-Portfolio Software Epos zur Selbstreflexion und Selbstorganisation des Studiums im Bachelor Biologie: Mitautor, 2012
- Erstellung eines studienbegleitenden Kompetenzportfolios in Mahara: Mitautor, 2011/2012
- Fragen entwickeln, Antworten geben im Stud.IP-Forum: Mitautor 2010/2011
- Umbau eines Praktikumsskripts in ein ILIAS-Lernmodul: Mitautor: 2010
- ILIAS-Tests als Vorlesungswiederholung und Vertiefung: Mitautor 2009/2010
- Online-Tutorium mit Diskussionsfourm: Mitautor 2009
- Erstellung eines gemeinsamen Glossars im WiKi: Mitautor 2008/2009

Lehrerfahrungen

Population Ecology (M.Sc.): Betreuung des Laborteils

Behavioral Ecology (M.Sc.): Betreuung des Laborteils

Advanced Statistics and Experimental Design (M.Sc.): Betreuung der Übung

Struktur und Funktion der Wirbellosen (B.Sc.): Betreuung des Laborteils

Grundkurs Ökologie (B.Sc.): Betreuung des Laborteils / Vorlesungsvertretung

Biologiedidaktik – Erstellung von Lehrvideos (M.Ed.): Betreuung/Mitleitung des Seminars

Charles Darwin – Der Einfluss seiner Theorien auf Wissenschaft und Gesellschaft (General Studies): Betreuung des begleitenden Online-Seminars

Evolution (B.Sc): Erstellung von zusätzlichem Online-Kursmaterial

Weitere Erfahrungen

Mitorganisator des 19. Graduiertentreffens der Fachgruppe Evolutionsbiologie der DZG 2014, Bremen

Mitbetreuung zahlreicher B.Sc. und M.Sc.Arbeiten