INFORMATION USE AND MEMORY FORMATION DURING FORAGING IN THE PARASITOID *NASONIA VITRIPENNIS*

MAREIKE KOPPIK

Universität Bremen*

Information use and memory formation during foraging in the parasitoid *Nasonia vitripennis*

Dissertation zur Erlangung des Doktorgrades

Dr. rer. nat.

an der Universität Bremen

Fachbereich 02, Biologie/Chemie

vorgelegt von

Mareike Koppik

Bremen, Juli 2014

1st **Reviewer:** Prof. Dr. Thomas S. Hoffmeister

2nd **Reviewer:** Dr. David M. Shuker

Table of contents

1

Summaries

1.1 Summary

The use of information is a successful mechanism in behavioral adaptations to changing environments. In order to adapt behavior to current conditions, animals have to constantly make various decisions, e.g. on foraging strategies. Parasitoid females have proven to be valuable model systems in studies of foraging decision making. **Chapter 2** covers theoretical models and empirical studies on three important foraging decisions in parasitoid females: 1) Where to search for hosts?, 2) When to leave a patch? and 3) What clutch size to produce? It is emphasized that, besides the information state, the physiological state of an individual may influence foraging decision making. Chapter 2 also deals with learning and memory formation, which is one key mechanism in host finding through information use in parasitoids.

Chapters 3-6 describe the experiments that have been conducted in order to investigate foraging decision making in the parasitoid *Nasonia vitripennis*. The aims of these experiments were 1) to disentangle the relative contributions of physiological state and information state to foraging decisions, 2) to elucidate the role of different habitat information in foraging decisions and 3) to get a detailed insight into associative learning and subsequent memory formation for host related odors in *N. vitripennis*.

One key parameter of foraging habitats of parasitic wasps is the quality of the host patches therein. Host patch quality is determined by the number and quality of hosts that can be found in a patch. Host quality has been demonstrated to influence the number of emerging offspring in the study species *N. vitripennis*. To confirm that varying offspring numbers result from varying clutch size decisions, and thus from the perception and use of host quality information by the female, the relative contributions of offspring mortality and clutch size decisions on final offspring production were investigated in **chapter 3**. It was confirmed that offspring mortality is negligible in live hosts of varying quality and that different offspring production resulted from female clutch size decisions and hence information use.

Host encounters can provide females with several valuable information. A female that encounters a host in the presence of a novel odor can use this information in later host finding decisions, as this odor may reliably indicate the presence of hosts in her current environment. The mechanisms behind this information use in parasitoids is often identified as associative learning. Yet, learning behavior and subsequent memory formation may differ considerably between and within species. Therefore, the specific dynamics of associative learning and memory formation in *N. vitripennis* were investigated in **chapter 4**. The results demonstrate two important facts about information use in host finding in *N. vitripennis*. Firstly, memory retention rate increased with the intensity of the host examination mode (conditioning treatments with varying host experience intensities), and host quality had no apparent influence on learning behavior. Secondly, females showed considerable intraspecific differences in their learning behavior, which may reflect the broad variety of hosts and host habitats that are used by *N. vitripennis*.

Host encounters and the quality of encountered hosts can also provide a female with valuable information about the quality of her habitat, which in turn should influence her clutch size and time allocation decisions. **Chapter 5** demonstrates that clutch size decisions in *N. vitripennis* were influenced by the information about previous host encounters (differing for different host examination modes) but not by the previous host quality. This bears resemblance to information use in host finding through associative learning and may suggest the use of the same pathway in initial information processing in these two decisions. The influence of information state could only be detected after correcting for the egg state of the females, as this seemed to be an important physiological state influencing clutch size decisions in *N. vitripennis* females.

To investigate the causes for the strong dependency of *N. vitripennis* females on their current egg state, egg maturation rate and the Lack Clutch Size in this species was analyzed in **chapter 6**. The results strongly suggested that females of *N. vitripennis* face transient (temporal) egg limitation, which makes egg-state dependent clutch size decisions an adaptive behavioral strategy according to theoretical models. Chapter 6 also shows that females of *N. vitripennis* displayed behavioral adaptations to transient egg limitation in their time allocation decisions. Females with low egg loads remained on the host most likely to mature and lay further eggs.

The present study gives a detailed insight into the foraging decisions made by *N. vitripennis* females, which are discussed in the light of physiological state and information state dependent decision making in **chapter 7**. It is concluded that females show various behavioral adaptations to transient egg limitation in their foraging decisions. Yet, information about habitat parameters are also used by the females to make clutch size and time allocation decisions. This information use shows intriguing parallels to information use in associative learning of host related odors. Thus, this suggests that initial information processing in both foraging decisions may use the same pathway.

1.2 Zusammenfassung

Verhaltensanpassungen an eine sich verändernde Umgebung können durch die Nutzung von Informationen über jene Umgebung erfolgen. Diese Verhaltensanpassungen erfordern das ständige Treffen von Entscheidungen über die angemessenen Verhaltensstrategien. Entscheidungsverhalten, besonders im Kontext von optimalem Suchverhalten, wurde wiederholt erfolgreich an parasitischen Wespen untersucht. **Kapitel 2** gibt einen Überblick über diese Untersuchungen – unter Einbezug von theoretischen als auch empirischen Studien – mit dem Fokus auf drei von zahlreichen Entscheidungen, die Weibchen parasitischer Wespen bei der Suche nach und der Ausbeutung von Wirten treffen müssen: 1) Wo wird nach Wirten gesucht?, 2) Wann wird ein gefundener Wirtspatch wieder verlassen? und 3) Wie viele Eier werden an einen einzelnen Wirt gelegt? Darüber hinaus wird in diesem Kapitel auch auf den Einfluss des physiologischen Status eines Weibchens auf ihr Entscheidungsverhalten eingegangen. Des Weiteren bietet Kapitel 2 einen kurzen Einblick in assoziatives Lernen. Dies hat sich als ein Schlüsselmechanismus zur Informationsnutzung bei der Wirtssuche von parasitischen Wespen erwiesen.

In den darauf folgenden Kapiteln 3-6 werden die einzelnen Experimente beschrieben, die der Untersuchung der Informationsnutzung bei der parasitischen Wespe *Nasonia vitripennis* dienten. Ziel dieser Experimente waren: 1) das klare Trennen von Entscheidungen, die auf dem physiologischen Status eines Weibchens beruhen, von solchen, die auf Informationsnutzung beruhen, 2) die Analyse von Informationen über Habitatparameter, die Einfluss auf die Entscheidung von Weibchen nehmen und 3) ein detaillierter Einblick in das assoziative Lernen von Gerüchen und die nachfolgende Gedächtnisbildung bei *N. vitripennis*.

Ein wichtiger Habitatparameter, der von parasitischen Wespen bei der Entscheidung über ihr Suchverhalten und die Ausbeutung von Wirten genutzt wird, ist die Qualität der Wirtspatches innerhalb des Habitats. Die Qualität dieser Patches wird von der Anzahl und Qualität der darin enthaltenen Wirte bestimmt. Die Qualität eines Wirtes beeinflusst die Anzahl der schlüpfenden Nachkommen bei *N. vitripennis*. Um zu klären, ob dies aus der Wahrnehmung der Wirtsqualität des Weibchens und der daraus resultierenden

Gelegegröße-Entscheidung resultiert, wurde der relative Beitrag von Mortalität der Nachkommen und Entscheidung der Weibchen zur Anzahl der Nachkommen untersucht (**Kapitel 3**). Die Experimente konnten bestätigen, dass die Mortalität der Nachkommen an lebenden Wirten vernachlässigbar ist. Tatsächlich ist die Wahrnehmung der Wirtsqualität des Weibchens – und die daraus resultierende Entscheidung – für die Anpassung der Anzahl der Nachkommen an die Wirtsqualität verantwortlich.

Begegnungen mit Wirten bieten Weibchen verschiedene wertvolle Informationen, die zu einer Verhaltensanpassung führen können. Die Geruchsumgebung, in der ein Wirt aufgefunden wird, kann ein Wegweiser für späteres Suchverhalten darstellen, da bei diesem Geruch wahrscheinlich – unter aktuellen Bedingungen – weitere Wirte aufgefunden werden können. Der Mechanismus, mit dem diese Information von parasitischen Wespen genutzt wird, ist gut untersucht. Häufig nutzen Weibchen assoziatives Lernen von neuen Gerüchen, um ihr Suchverhalten zu optimieren. Allerdings unterscheiden sich das Lernverhalten und die anschließende Gedächtnisbildung erheblich zwischen den verschiedenen Arten. Daher befasst sich **Kapitel 4** mit dem spezifischen Lernverhalten bei *N. vitripennis*, aus dem zwei Hauptergebnisse zu nennen sind. Erstens, mit zunehmender Intensität der Wirtsbegegnung (bis zu welchem Grad dieser von dem Weibchen untersucht werden durfte) während der Konditionierung stieg auch die spätere Rate der Erinnerung der Weibchen für den assoziierten Geruch. Die Qualität des erfahrenen Wirts hatte darauf keinen Einfluss. Zweitens, es konnten erhebliche Unterschiede im Lernverhalten zwischen den Weibchen festgestellt werden, diese Unterschiede könnten dem breiten Wirtsspektrum und dem daraus resultierendem breiten Spektrum von Wirtshabitaten geschuldet sein.

Wirtsbegegnungen und die Qualität dieser Wirte gibt zudem Informationen über die Qualität des gesamten Habitats, diese Habitatqualität wiederum sollte einen Einfluss auf die Gelegegröße und die Zeitallokation der Weibchen haben. In **Kapitel 5** wird gezeigt, dass die Gelegegröße-Entscheidungen von Weibchen tatsächlich von vorangegangenen Wirtsbegegnungen beeinflusst werden (genauer von dem Grad zu welchem das Weibchen den Wirt untersuchen durfte). Allerdings zeigte sich auch hier kein Einfluss der Qualität des vorangegangenen Wirts. Diese Ergebnisse weisen erstaunliche Parallelen zu den Versuchen zum assoziativen Lernen auf. Diese Parallelen könnten darauf hindeuten, dass zumindest das erste Verarbeiten von Informationen über denselben Signalweg verläuft wie beim assoziativen Lernen. Der Einfluss von Informationen konnte nur nach Einbezug des Eivorrats der Weibchen gezeigt werden, da dieser physiologische Status einen wichtigen Einfluss auf die Entscheidungen der Weibchen hatte.

Mögliche Ursachen für diesen starken Einfluss des Eivorrats werden in **Kapitel 6** betrachtet. Basierend auf der Eireifungsrate der Weibchen und der Kapazität der Wirte bezüglich der optimalen Anzahl von Wespenlarven, kann gefolgert werden, dass Weibchen zumindest zeitweise durch ihren Eivorrat in ihrer Reproduktion limitiert sind. Theoretische Modelle zeigen, dass unter diesen Voraussetzungen Verhaltensanpassungen an den aktuellen Eivorrat eine adaptive Strategie darstellen können. Des Weiteren konnte in diesem Kapitel gezeigt werden, dass Weibchen in ihrer Zeitallokation eine spezielle Anpassung an Eilimitierung zeigen. Bei sehr niedrigem Eivorrat verblieben die Weibchen auf dem Wirt, vermutlich um Eier nachzureifen und legen zu können.

Die vorliegende Arbeit gibt detaillierte Einblicke in die Such- und Eiablage-Entscheidungen der parasitischen Wespe *N. vitripennis*, welche unter Berücksichtigung der Einflüsse des physiologisches Status und der Informationsnutzung in **Kapitel 7** diskutiert werden. Eine Schlussfolgerung dieses Kapitels ist, dass *N. vitripennis* verschiedenste Verhaltensanpassungen an temporäre Eilimitierung zeigt. Des Weiteren nutzen Weibchen Informationen über Habitatparameter, um Eiablage- und Zeitallokations-Entscheidungen zu treffen. Diese Informationsnutzung weist bemerkenswerte Parallelen zu assoziativem Lernen von Gerüchen auf. Dies weist darauf hin, dass in beiden Prozessen derselbe Signalweg bei der Informationsverarbeitung verwendet werden könnte.

2 General introduction

Adapting behavior to changing environments often requires the integration of information about current conditions into the decision making process (Dall *et al.* 2005). Optimal decision making in animals, foraging for food or hosts, is dealt with in Optimal Foraging Theory (Krebs 1977). Here, animals would need to know the current overall quality of their foraging habitat, in order to make optimal foraging decisions in variable habitats. Since animals are not omniscient regarding their environment, the overall habitat quality can only be estimated. These estimations can be carried out by using previous experiences in the habitat that may indicate current conditions. Parasitoids play a huge role in empirical studies about information use in foraging decision making (van Alpen, Bernstein & Driessen 2003), because of the close link between a female's foraging success and her lifetime fitness gain (Godfray 1994). These studies have demonstrated that parasitoid females use previous experiences to adjust their current foraging behavior (see Wajnberg 2006 for a review). Thus, it can be hypothesized that they use information gathered during foraging to estimate overall habitat quality. The mechanisms behind this information use, though, remain to be investigated.

Well studied, in terms of mechanisms behind information use in parasitoid females, is associative learning of host related chemical cues facilitating host finding (see Hoedjes *et al.* 2011for a review). An emerging model organism among parasitic wasps is the parasitoid *Nasonia vitripennis* (Werren & Loehlin 2009a), being the first parasitoid wasp with a completely sequenced genome (Werren *et al.* 2010). The ability of associative learning had already been demonstrated in this species (Oliai & King 2000; Baeder & King 2004; Schurmann *et al.* 2009). The aim of the present study was therefore to use the parasitoid *N. vitripennis* as a study system to investigate the mechanisms behind information use in foraging decisions in parasitoids. A special emphasis laid on the question whether learning and the subsequent formation of memory is involved in information processing of habitat information during foraging in parasitoids. Therefore, the following three main objectives of the present study can be named:

1) Identifying the habitat parameters that are perceived and used by *N. vitripennis* females to estimate overall habitat quality. To study information use, one important aspect was to clearly disentangle between behavioral adaptations that depend on the physiological state and those that dependent on the information state of a female.

2) Investigating the temporal dynamics of memory formation after associative learning in *N. vitripennis* to identify memory phases that might be used in information processing in habitat quality estimation, and identifying memory inhibitors for the relevant memory phases.

3) Elucidating the role of memory formation in the estimation of habitat quality in *N. vitripennis* by trying to inhibit the memory formation for single habitat experiences and analyzing the resulting habitat quality estimation.

During the course of this study, it became apparent that females of *N. vitripennis* quickly consolidate memory during the parasitization of a host in associative learning tasks, preventing the use of anesthesia to delete memory (see 2.2). No memory inhibitor that could inhibit the formation of the following medium term memory phase (MTM I, Figure 2.4) was found, hampering further studies of the role of learning and memory in habitat quality estimation. At the same time, experiments demonstrated interesting behavioral adaptations to the physiological state of a female, in addition to information use. Therefore, the following thesis contains experiments on learning behavior in *N. vitripennis* due to the original objectives of the study. But, due to technical reasons that impeded the pursuit of the original problem, the thesis will concentrate on the first objective and the successfully tackled questions therein without any further reference to the original problem. Yet, experiments revealed intriguing parallels between properties of associative learning and properties of information use in foraging decisions, which are discussed in chapters 5 and 7 in detail. These parallels suggest that at least initial information processing may use the same pathway in associative learning and in the estimation of habitat quality. Thus, while the present study did not answer the question whether learning and memory is involved in habitat quality estimation in parasitic wasps, it indicates that further research in this direction is worthwhile.

2.1 Information use and decision making during foraging in parasitoids

A parasitoid female foraging for hosts has to make various decisions (Figure 2.1) – on the host as well as on the patch level (Charnov & Skinner 1988). The close link between oviposition decisions and fitness in parasitoids makes them ideal study systems (Godfray 1994), and they thus have gained a lot of attention in theoretical as well as empirical studies (e.g. Rosenheim & Rosen 1991; Roitberg *et al.* 1993; Heimpel, Rosenheim & Mangel 1996; Roitberg, Zimmermann & Hoffmeister 2010; Thiel 2011). Understanding decision making often requires identifying the underlying costs and benefits of different options. In parasitoid oviposition decisions two major costs have been identified – time and eggs – but the relative contributions of the two often remain unclear (Rosenheim *et al.* 2008). Females that encounter fewer hosts than they have eggs to deposit, are mainly limited by the time they have to successfully locate and parasitize these hosts. Time limitation has been argued to be the most likely outcome of the evolution of egg maturation rate and egg supply in insects (Sevenster, Ellers & Driessen 1998; Ellers, Sevenster & Driessen 2000). Empirical studies have demonstrated that foraging behavior of many parasitoid species is in accordance with models assuming time limitation (see Wajnberg 2006 for a review). When the number of hosts females are able to find and parasitize during their lifetime exceeds the number of eggs they have available, they are rather limited by their egg supply. Another group of biologists has argued that evolution should favor an egg maturation rate that leads to egg limitation in at least a fraction of females within one generation (Rosenheim 1996; Rosenheim 2011). Field studies in some species were found to support this hypothesis (Heimpel, Mangel & Rosenheim 1998; Heimpel & Rosenheim 1998; Casas *et al.* 2000; Rosenheim *et al.* 2008; Segoli & Rosenheim 2013). Under natural conditions, these two limitations may rather be on a continuum than strictly separated categories and they may even be different depending on the time scale. While synovigenic parasitoids – maturing eggs throughout their lifetime – might not be limited by their lifetime egg supply, they may well be temporarily egg limited (transient egg limitation) depending on their host encounter and egg maturation rate (Charnov & Skinner 1988; Casas *et al.* 2000; Rosenheim, Heimpel & Mangel 2000). Thus, the egg maturation rate and the possible oviposition opportunities of *Nasonia vitripennis* were estimated in the present study (chapter 6) to understand the limiting factors of reproduction in this parasitoid.

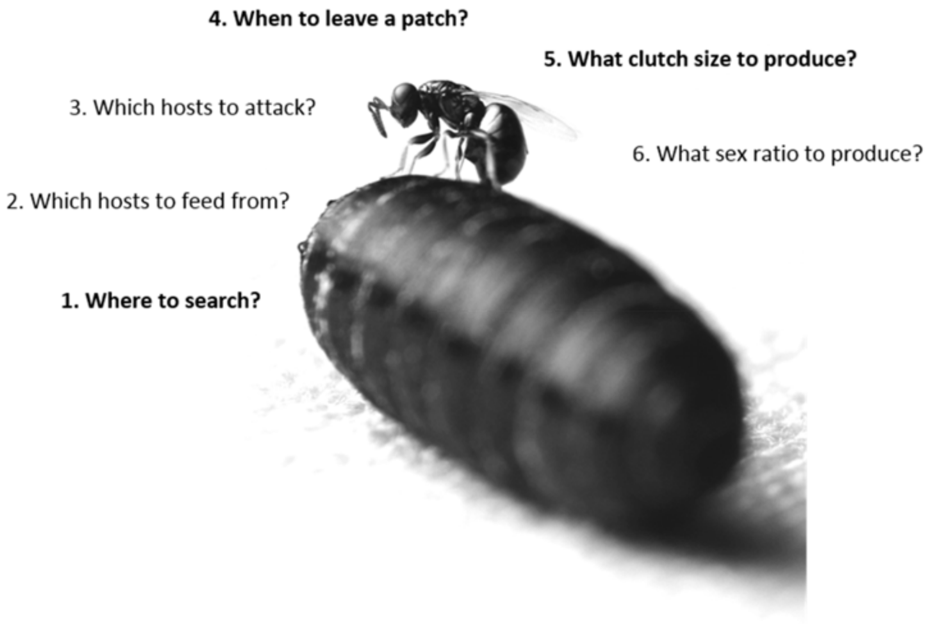

Figure 2.1: Foraging decisions in parasitoid females (adapted from Charnov & Skinner 1988), decisions in bold letters were investigated in the parasitoid *Nasonia vitripennis* within the present study

The time available for foraging and resources available for egg laying are limiting a parasitoid female’s lifetime reproductive success. Foraging decisions (Figure 2.1) influence the reproductive success of a female, since the efficient localization and exploitation of hosts are directly connected to the number and fitness of offspring a female can produce. In variable environments containing patchily distributed hosts, the use of information on various habitat parameters can optimize foraging behavior over time (McNamara & Houston 1985). Yet, decisions may additionally be influenced by a forager’s physiological state (Mangel 1989). To elucidate the contributions of physiological state and information state – the latter based on various habitat parameters – to foraging decision making, the following three foraging decisions were investigated in the parasitoid *N. vitripennis*.

Host finding (Where to search?)

Efficiently locating (concealed) hosts in the environment is a major challenge parasitoid females have to face during foraging (Godfray 1994, p 26). Besides innate responses to host cues, females may also use information about host related cues gained through associative learning to improve host searching behavior (see chapter 2.2.1).

Time allocation (When to leave a patch?)

As a female parasitoid exploits a patch of hosts, the number of unparasitized hosts and thus patch quality decreases with every host she parasitizes. Hence, over time her oviposition rate, thus her rate of fitness gain, declines. In time limited parasitoids it has been argued that females should be selected to maximize their rate of fitness gain (Wajnberg 2006). Rate maximization in environments containing depletable patches has been modeled for predators with Charnov's Marginal Value Theorem (Charnov 1976). Predictions derived from this model often show a good qualitative match with the behavior of time limited parasitoids foraging for hosts (see Wajnberg 2006 for a review). According to the Marginal Value Theorem, the female should leave a patch when its gain rate on the patch drops below the average gain rate in the habitat (Charnov 1976). Thus, patch leaving depends on the quality of the current patch compared to the quality of the overall habitat. The information on habitat quality has to be perceived, sampled and stored, and finally integrated into foraging decisions. Additionally, a forager's physiological state may influence the decision when to leave a patch, which may explain quantitative deviations from the model predictions. It has been shown earlier that low egg loads can lead to lower patch exploitation and earlier patch leaving in a parasitoid, most likely to avoid egg depletion (Outreman *et al.* 2005). In contrast to this observation, a model by Charnov and Skinner on patch time allocation in gregarious parasitoids predicts that females may remain on the host to mature and lay additional eggs when being egg depleted (Charnov & Skinner 1988). Yet, the amount of time a female should remain on the host is again dependent on the quality of the overall habitat. Thus, females also should integrate information about the habitat into the decision how much time to wait on the host. These predictions have rarely been tested to date. For *N. vitripennis* Edwards (1954b) reported the anecdotal observation that females remain on the host after an oviposition bout especially at low egg loads. In the present study (chapter 6) it has been tested, whether time allocation in *N. vitripennis* is in accordance with the predictions of Charnov and Skinner that female parasitoids may remain on a host at low egg loads and that this waiting time is dependent on habitat parameters (Charnov & Skinner 1988).

Clutch size (What clutch size to produce?)

Females of gregarious parasitoid species, i.e. species that provide each host with more than a single egg, not only have to decide which hosts to attack, but also how many eggs to lay into a host accepted for oviposition. Since hosts represent a limited resource for the developing offspring, competition between offspring feeding from the same host may occur (Godfray 1994, pp 257-258). This competition leads to a decrease in survival and/or fitness of the offspring with increasing clutch size (Hardy, Griffiths & Godfray 1992; Withers, Madie & Harris 1997; Desouhant *et al.* 2000; Zaviezo & Mills 2000; Hoffmeister & Rohlfs 2001; Pexton *et al.* 2009). From an offspring's point of view, single egg clutches would therefore be optimal, given there are no Allee effects that lead to an increase in individual fitness with increasing conspecific density (Stephens, Sutherland & Freckleton 1999). Yet, from a female's point of view, larger clutches mean more offspring and therefore a higher fitness return. The single host optimum for a female can be found using the rationale of Lack Clutch Size (Lack 1947). In insects it has been adapted to find the clutch size that gives the highest fitness return for the ovipositing female (Figure 2.2), e.g. by incorporating the typical size-fitness relationship in insect females (Mangel, Rosenheim & Adler 1994). Additionally, the Lack Clutch Size depends on the quality of the host, as this usually determines the resources available for competing siblings (Charnov & Skinner 1984).

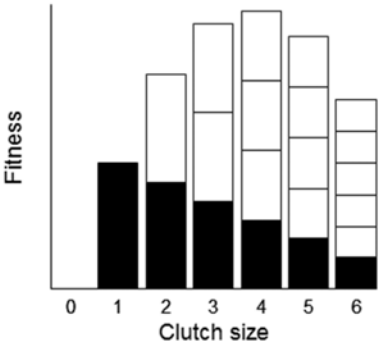

Figure 2.2 Graphical representation of the Lack Clutch Size in parasitoids (adapted from Godfray 1994, p 100). With increasing clutch size fitness of an individual offspring (height of the black bars) decreases – e.g. due to decreased survival probability or fecundity –, while total fitness gain for the ovipositing female (total height of the bars) still increases – due to increasing numbers of offspring - until optimal clutch size (here the Lack Clutch Size = 4) is reached

A study that estimated the Lack Clutch Size in two parasitoid species and compared those values to clutch sizes reported for those species, demonstrated that the Lack Clutch Size does not match well with the average clutch sizes females produce, but seems to set an upper limit for clutch size in these species (Charnov & Skinner 1984). It has been argued that the Lack Clutch Size should only be laid if there are no fitness costs associated to egg laying (Godfray 1994) – either because current reproductive effort does not influence future reproduction or because there is no future reproduction. Usually there are costs connected to egg laying, with time and eggs invested in oviposition representing two of them (see above). Laying an additional egg to the clutch costs the same amount of time and egg resources independent of the current clutch size while the fitness return per egg decreases with increasing clutch size (Figure 2.2). Therefore, at a certain point the female would obtain a higher fitness return from laying her eggs into – or allocating her time to – a new clutch. Since clutch size can be treated as an optimal foraging problem (Iwasa, Suzuki & Matsuda 1984; Skinner 1985; Mangel 1987), this point should be dependent on the current host quality and the overall habitat quality. Lower host quality and higher overall habitat quality should lead to a lower optimal clutch size for the female. Studies on clutch size decisions in parasitoid females under varying habitat qualities are rare (but see Rosenheim & Rosen 1991; Bezemer & Mills 2003), while there is a number of studies on the influence of egg load on clutch size in insects (see Minkenberg, Tatar & Rosenheim 1992 for a review). One reason for this might be that it is often difficult to disentangle egg load and experience (with varying habitat quality) (Rosenheim & Rosen 1991). Disentangling the contribution of physiological state and information state would help to understand the role of information use in clutch size decisions in gregarious parasitoids. Therefore, the present study aimed at investigating the role of physiological state and information state (chapters 3 and 5) on clutch size decisions in *N. vitripennis* females.

2.2 Learning and memory

Parasitoid females often use information on host (or host-habitat) related chemical cues to efficiently locate their hosts (Godfray 1994). If this is not an innate response, the mechanism behind this information use is often identified as associative learning and the formation of memory (Godfray & Waage 1988; Hoedjes *et al.* 2011) (see 2.2.2).

Learning can be defined as a change in cognitive state due to experience, but this change in cognitive state can usually only be observed through changes in behavior (Shettleworth 2010, pp 98/99). Learning allows for behavioral plasticity in order to adapt to changing environmental conditions (Dukas 1998b, p 129). This adaptation can lead to a high fitness benefit for the learning individual (Papaj & Vet 1990; Steidle 1998; Dukas & Bernays 2000; Egas & Sabelis 2001; Raine & Chittka 2008; Cole *et al.* 2012), but learning is also connected to costs (Burns, Foucaud & Mery 2011; Cole *et al.* 2012). Costs of learning behavior can be divided into two main groups a) constitutive (indirect) costs: e.g. physiological costs for creation and maintenance of the neural requirements of learning, trade-offs with life-history traits (Mery & Kawecki 2003; Burger *et al.* 2008; Snell-Rood, Davidowitz & Papaj 2011) and b) operating (direct) costs: e.g. metabolic costs of learning (Mery & Kawecki 2004; Mery & Kawecki 2005) as well as costs connected to errors that occur during learning leading to the use of irrelevant cues.

According to theoretical models, learning pays off when changes in the environment occur within an intermediate time scale, e.g. when variability in conditions is high between but low within generations (Stephens 1991; Dukas 1998b; Eliassen *et al.* 2009). If habitat conditions or food sources are rather stable over many generations (e.g. in specialists compared to generalists) behavioral plasticity and therefore learning is not necessary, instead an innate behavioral response can be adaptive (Laverty & Plowright 1988). In habitats with frequent changes of environmental conditions within a generation, learning might not pay off as well because of the low predictive value of the current situation for future conditions (Dukas 1998b). Hence, under intermediate variability, e.g. when conditions change between generations but changes are not too frequent within generations, learning is highly favorable. Here, learning of current conditions that do not change during an individual's lifetime and are of high predictive value can lead to an improved performance (McNamara & Houston 1985).

While learning is the acquisition of information, memory formation and retention actually allow to use these information and change behavior due to experience (Shettleworth 2010, p 210). Memory can be roughly separated into three phases: short term, medium term and long term. These memory phases are named after the relative time they last, but are usually identified by the way their formation can be disrupted (Dezazzo & Tully 1995). Short term memory (or anesthesia sensitive memory) usually lasts for a few seconds or minutes and can be disrupted by several forms of anesthesia (Xia, Feng & Guo 1999). The memory form following short term memory is called medium term or intermediate memory (or anesthesia resistant memory) and usually lasts minutes to hours or at most a few days. It is defined as the memory stage that is anesthesia resistant but does not require transcription (Dezazzo & Tully 1995). Thus, medium term memory is not susceptible to transcription inhibitors, but can often be disrupted by translation inhibitors (Xia, Feng & Guo 1998; Schurmann *et al.* 2009). Long term memory lasts from several hours up to an individual's lifetime. Long term memory is characterized by its dependency on protein biosynthesis (Dezazzo & Tully 1995). In contrast to medium term memory it is dependent on the translation and transcription of genes, therefore the formation of a long term memory can be blocked by applying transcription inhibitors (Tully *et al.* 1994; Schurmann *et al.* 2012). Usually an animal is able to form different kinds of memories differing in their specific physiological costs and the length of time information can be recollected by the individual. Therefore memory allows for a more fine-tuned use of experience. Cues that are highly reliable and might predict future conditions for a long period of time should be stored in a more costly but stable long-lasting form of memory (Eliassen *et al.* 2009). Highly reliable cues predicting conditions only for short period of time, on the other hand, might be stored in less costly, stable and shorter memory forms (Eliassen *et al.* 2009). Rather than seeing forgetting as a failure of the neuronal system to keep information, forgetting can actually be seen as an adaptive mechanism avoiding the costs of using cues that have become irrelevant (Dunlap *et al.* 2009; Ferrari *et al.* 2010). Cues that are rather unreliable might be first stored in an unstable memory that easily vanishes. If these cues are encountered several times in a quick succession (making them more reliable), this information is still stored and can then be transferred to more stable forms of memory. If the cue is not encountered anymore the information quickly vanishes and the costs of using an irrelevant cue is comparatively low. Considering the different costs and benefits and the high plasticity in memory, even within a species, it is not surprising that studies on animal cognition have revealed high variation in cognitive abilities between species (Healy *et al.* 2009). This variability has led to the question how the ecology of a species influenced the evolution of its learning behavior (Healy *et al.* 2009). Because of the close link between host finding ability and fitness in parasitoids, along with their broad ecological diversity, parasitoids have become promising model systems in studies on the adaptive value of learning and the ecological background shaping different learning strategies (Hoedjes *et al.* 2011). Information use in host finding through associative learning has been well studied in parasitoids with a recent focus on interspecific differences (Bleeker *et al.* 2006; Smid *et al.* 2007; van den Berg *et al.* 2010; Hoedjes *et al.* 2012; Hoedjes & Smid 2014). Differences within a species in this kind of information use during foraging has received less attention (but see van den Berg *et al.* 2011; Kruidhof *et al.* 2012; Thiel, Schlake & Kosior 2013). Thorough work on intraspecific variability in learning behavior in *Drosophila melanogaster* (Diptera: Drosophilidae) has demonstrated the usefulness of such studies in order to estimate costs and benefits of learning behavior (see Kawecki 2010 for a review). Therefore, intraspecific variability in associative learning in the parasitic wasp *Nasonia vitripennis* has been a focus within the present study (chapter 4).

2.2.1 Associative learning and memory formation in parasitoids

Reliable cues that indicate the presence of suitable hosts usually derive directly from the hosts and, as hosts should be selected to conceal their presence, are hard to detect by their parasitoids (Vet, Wäckers & Dicke 1991). One solution to overcome this problem is to learn to associate those reliable, host derived cues with cues easier to detect from a distance (Vet, Wäckers & Dicke 1991). Many parasitoid females show the ability of learning of host (or host-habitat) related chemical cues (Lewis & Takasu 1990; Kaiser *et al.* 2003; Takasu & Lewis 2003; Bleeker *et al.* 2006; Muller *et al.* 2006; Smid *et al.* 2007; Schurmann *et al.* 2009; Kruidhof *et al.* 2012; Schurmann *et al.* 2012; Thiel, Schlake & Kosior

2013). It has been demonstrated that learning of host associated cues can facilitate host finding ability of a parasitoid and therefore increase its fitness (Papaj & Vet 1990).

Learning experiments in parasitoids often make use of classical (or Pavlovian) conditioning (Figure 2.3). The ability of associative learning in *N. vitripennis* has been demonstrated for males and females. When trained, males are able to associate the presence of a female with various colors (Baeder & King 2004). Females can learn to associate the presence of a host or food (honey) with a color (Oliai & King 2000) and are able to associate the presence of hosts with an odor (Schurmann *et al.* 2009; Hoedjes *et al.* 2012; Schurmann *et al.* 2012; Hoedjes & Smid 2014).

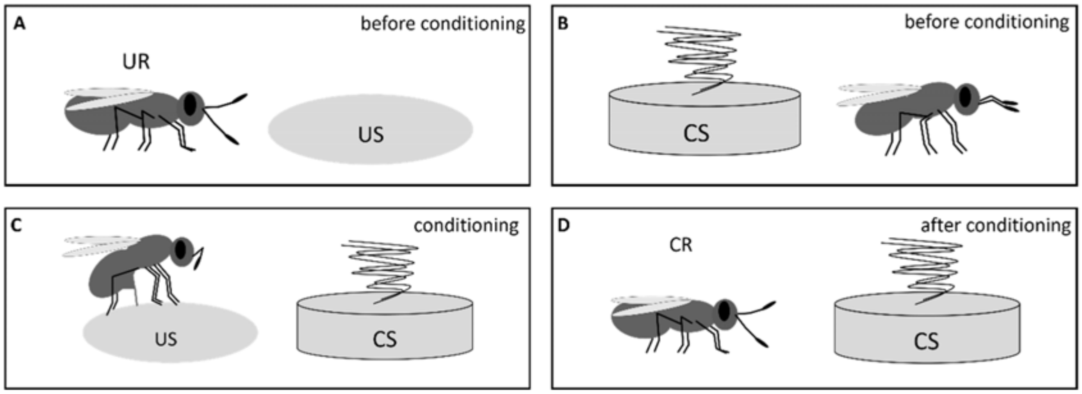

Figure 2.3: Classical conditioning of *Nasonia vitripennis* females. Before conditioning the presence of a host (US, unconditioned stimulus) elicits searching behavior (UR, unconditioned response) and, if a host is found, subsequent oviposition behaviors **(A)**, while a novel odor (CS, conditioned stimulus) does not elicit any of the former described behaviors **(B)**. During the conditioning females are exposed to the host (US) and the neutral odor (CS) simultaneously such that they learn to associate the presence of a host with the odor **(C)**. This association leads to the initiation of searching behavior (CR, conditioned response) when the odor (CS) is encountered after conditioning **(D)**.

Many studies on parasitoid learning behavior test for associative learning of novel odors (e.g. Dejong & Kaiser 1992; Kaiser *et al.* 2003; Schurmann *et al.* 2009; Thiel, Schlake & Kosior 2013). To analyze learning and the formation of memory, a female's odor preference is tested in e.g. a wind-tunnel (e.g. Kruidhof *et al.* 2012), a Y-tube olfactometer (e.g. Thiel, Schlake & Kosior 2013) or a four-chamber olfactometer (e.g. Muller *et al.* 2006). All these experimental set-ups test for the orientation of a female towards the learned odor either by flight or walk. In the field this behavior can help females to optimize their host searching behavior (Papaj & Vet 1990; Steidle 1998).

Though many parasitoid females show the ability of associative learning, the temporal memory dynamics differ widely between species (Hoedjes *et al.* 2011). In *N. vitripennis* distinct memory phases have already been identified (Figure 2.4), and studies have demonstrated that females of closely related *Nasonia* species differ in their learning behavior and memory retention (Hoedjes *et al.* 2012; Hoedjes & Smid 2014). Intraspecific differences, though, have not been addressed yet. Hence, chapter 4 built on the knowledge available on *N. vitripennis* learning behavior (Schurmann *et al.* 2009; Schurmann *et al.* 2012) and investigated intraspecific variability in learning of host related odors – as one aspect of information use during foraging in parasitoids.

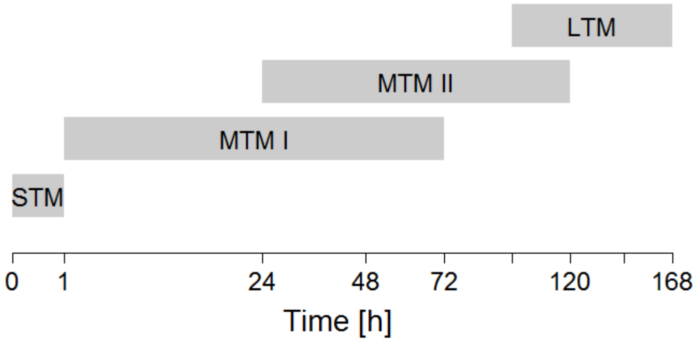

Figure 2.4: Temporal memory dynamics after learning in *Nasonia vitripennis* (adapted from Schurmann *et al.* 2012)
Abbreviations:
STM: short term memory;
MTM: medium term memory;
LTM: long term memory

2.3 Biology of the parasitic wasp *Nasonia vitripennis*

Nasonia vitripennis (Walker) (Hymenoptera: Pteromalidae) and its three closely related sister species *N. longicornis*, *N. giraulti*, and *N. oneida* are parasitic chalcid wasps within the family of Pteromalidae. While its sister species can only be found in North America (Darling & Werren 1990; Raychoudhury *et al.* 2010), *N. vitripennis* is distributed worldwide probably because it parasitizes pupae of human associated flies (Werren & Loehlin 2009a).

Male and female morphology especially differs in wing size (Figure 2.5). Females are fully winged with their wings extending over the end of the abdomen and are well able to fly. In contrast, the wings of males are much smaller never reaching the end of the abdomen, and males cannot fly. The size of females usually ranges between 1-3 mm while males are often slightly smaller (Whiting 1967). Developmental time depends on host species and temperature (Voss, Spafford & Dadour 2010). At 25 °C offspring emerges approximately 14 days after parasitization of the host (Whiting 1967). There are no field data available on the life span of *N. vitripennis*, but under laboratory conditions female life span is reported to be around 22 days in the absence of hosts (and fed sucrose solution) and 15 days in the presence of hosts (Davies 1975); under starvation males and females only live for a few days (King & Hopkins 1963).

Figure 2.5 Males (**bottom**) and females (**top**) of *Nasonia vitripennis* can be easily distinguished by the size of their wings

Hosts

N. vitripennis is a generalist species parasitizing a wide range of cyclorraphous Diptera (Whiting 1967). The hosts can mainly be found in birds' nests and on carcasses (Abraham 1985; Voss, Spafford & Dadour 2009). In birds' nests parasitic as well as necrophagous flies that serve as hosts for *N. vitripennis* can be found (Peters 2010). Abundance of ectoparasitic flies feeding on nestlings may vary greatly due to varying nestling abundances, resulting in differing host densities for *N. vitripennis* depending on the season and year (Daoust *et al.* 2012). But also abundance and composition of necrophagous fly species varies between seasons (Brundage, Bros & Honda 2011; Castro *et al.* 2012). This variation of host abundance and quality in time makes behavioral adaptation to current conditions by *N. vitripennis* females likely. It has already been shown that females of *N. vitripennis* change their foraging behavior with varying host densities (Wylie 1966) and distributions (Jones & Turner 1987) though the mechanisms (information based or state-dependent) leading to these behavioral adaptations remained unclear.

Reproductive behavior

N. vitripennis is a gregarious species, laying many eggs onto one host (Whiting 1967). The number of eggs laid is highly dependent on the host, with large species or specimen usually receiving more eggs than smaller ones (Wylie 1967; Peters 2010). They are endo-ectoparasitoids laying their eggs onto the host pupa but within the host puparium (Edwards 1954b). Females are highly synovigenic, they hatch with no mature eggs inside their ovaries and mature eggs throughout their lifetime (Edwards 1954a). Females need host feeding to gain nutrients, mainly proteins, for continuous egg maturation (Edwards 1954a; Richard & Casas 2012). Since male offspring is not able to disperse (at least not by flight) after emergence to find mates, male offspring of *N. vitripennis* often faces Local Mate Competition (LMC, Hamilton 1967). On single foundress host patches males compete only with their brothers for mating opportunities, leading to a highly female biased sex ratio being optimal (Werren 1980). Many studies have shown that *N. vitripennis* females adjust their sex ratio according to the perceived level of LMC, in the laboratory (Werren 1984; Shuker & West 2004; Shuker *et al.* 2007) as well as in nature (Molbo & Parker 1996; Burton-Chellew *et al.* 2008; Grillenberger *et al.* 2008).

Sequence of parasitizing behavior

Parasitization of a host by females of *N. vitripennis* consists of several phases that usually occur in a fixed order (Edwards 1954b). Distinct phases of the oviposition behavior are used as training procedures in experiments on associative learning (chapter 4) and are also analyzed in terms of time allocation (chapter 6). Therefore, a short description of female oviposition behavior adapted from Edwards (1954b) is given below:

<u>Host encounter:</u>

N. vitripennis is believed to have only limited visual abilities. Therefore females most likely orient upon chemical cues when locating an area containing hosts. Only in very close distance (2-3 mm), females might also use visual cues to orient towards a host (Edwards 1954b). Upon encountering a possible host (Figure 2.6) females usually quickly examine it with their antennae before they climb onto the host for a closer examination.

Figure 2.6 Female encountering a host puparium (*Calliphora vomitoria*)

<u>Drumming:</u>

After climbing onto the host, females start a thorough examination of the host's surface. They quickly walk over the host's surface and closely examine it through rapid alternating movements of their antennae, the so called Drumming phase (Figure 2.7). Most likely females now gather information about the size (Wylie 1967) and probably species of the host (through chemical cues on the host's surface).

Figure 2.7 Female on a host puparium during the Drumming phase

Tapping:

After exploring the host, females start searching for a drilling site. They bend their body such that the tip of the abdomen examines the host surface (Figure 2.8). The so called Tapping always precedes drilling and is used by the female to locate a good drilling site, which can be the drilling hole of another female, her own drilling hole that was made during a previous oviposition bout or – in unparasitized hosts – at an intersegmental notch of the host puparium (Whiting 1967). Once the ovipositor is positioned, the drilling phase starts.


Figure 2.8 Female in Tapping position to examine the host's surface with the tip of her abdomen

Drilling:

During the drilling phase the female drills a hole into the host puparium (Figure 2.9). After drilling has been successful the ovipositor is fully inserted into the host and the female is now able to examine the content of the host puparium. Here she might gather additional information about the status of the host (e.g. species, parasitization status, health and age), since many parasitoid have chemical receptors on the tip of their ovipositor (Quicke 1997, pp 189/190). She immediately stings the host pupae to inject a venom (Ratcliffe & King 1967) that arrests the host's development and alters its metabolism (Rivers & Denlinger 1994a; Rivers & Denlinger 1994b; Rivers & Denlinger 1995b) such that it is more suitable for the development of her offspring.


Figure 2.9 Female during drilling into a host puparium

Oviposition:

During oviposition a female lays her eggs onto the surface of the host pupa. The ovipositor is completely inserted into the host puparium for most of the time (Figure 2.10) and the female lays her eggs in a circle around the stinging site (Edwards 1954b). With the bare eye ovipositing and building a feeding tube cannot be distinguished.


Figure 2.10 Female with her ovipositor fully inserted into the puparium

Building feeding tube:

N. vitripennis females are concurrent host feeders, feeding from the same hosts they lay eggs onto. While a female builds a feeding tube, the ovipositor is more or less completely inserted into the host puparium (as during oviposition). She excretes a fluid that hardens and slowly builds up a tube that leads from the host's body to the outside. Within this feeding tube the host's body fluids rise up, such that the female can consume it.

Host Feeding:

After building a feeding tube, the female turns to face the feeding tube and starts drinking from the host's body fluids. The female's antennae are usually bend towards the host's surface during this behavior (Figure 2.11), such that it can be easily distinguished from resting. Building of a feeding tube and feeding from a host might occur repeatedly on the same host. Especially the proteins gained from host feeding are essential for the females to mature their relatively large protein rich eggs (Edwards 1954a; Richard & Casas 2012).

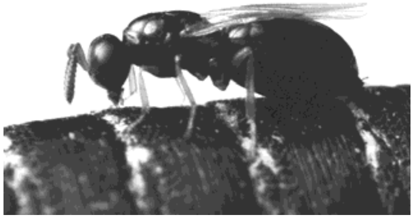

Figure 2.11 Female during host feeding

Resting on the host:

Oviposition behavior is often disrupted by longer resting periods, in which the female might engage in grooming but usually sits still on the host with her antenna resting in a straight line from her body (Figure 2.12). The anecdotal observation of long resting periods between oviposition bouts has been reported for females with low egg loads by Edwards (1954b).

Figure 2.12 Female resting on the host puparium

3 Adaptive decision making or differential mortality: what causes offspring emergence in a gregarious parasitoid?

Mareike Koppik, Andra Thiel & Thomas S. Hoffmeister*

Institute of Ecology, FB2, University of Bremen, Leobener Strasse, Bremen 28359, Germany

*Correspondence: E-mail: hoffmeister@uni-bremen.de

Short title: Clutch size or mortality driven offspring production?

Key words: *Nasonia vitripennis*, clutch size, egg allocation, oviposition decisions, developmental mortality, larval competition, offspring survival, Hymenoptera, Pteromalidae

Published in *Entomologia Experimentalis et Applicata* 150(3): 208-216,
doi: 10.1111/eea.12154

3.1 Abstract

Hosts represent a limited resource for the developing offspring of parasitic insects laying eggs in or on spatially discrete resources like fruits, seeds, or other insects. The quality of hosts differs with respect to the value and amount of resources they provide for the feeding larvae. Accordingly, the size of a clutch of eggs laid on a given host should be a function of host quality, because severe competition between developing larvae can lead to increased mortality and/or decreased size of the offspring, both causing a fitness loss for the offspring and the mother. Therefore, females should be selected for the ability to estimate host quality and to adjust their clutch size accordingly. Using the parasitic wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) this study investigated the respective contribution of developmental mortality of offspring vs. the clutch size decision of the mother as a determinant of final offspring emergence per host. Additionally, taking offspring size into account, the study examined the fitness consequences of female oviposition decisions. Developmental mortality was very low in all quality classes of hosts except previously frozen and thus dead host pupae. Females laid reduced clutch sizes on dead, previously parasitized, and smaller hosts. In contrast to offspring number, offspring size did not differ between host qualities. We conclude that females are able to sense the quality of a host and adjust the number of eggs they lay to mitigate larval competition.

3.2 Introduction

In insects with a parasitoid reproductive mode (sensu Price 1980), larvae develop destructively on spatially discrete and limited resources, which may be fruits, seeds, or other insects' bodies. This leads to a strong correlation between resource quality and fitness of the developing larva. In contrast to solitary species, where only a single offspring per host can survive and develop, gregarious offspring may share the host with their siblings and sometimes even with the offspring of conspecific females (Godfray 1994). Depending on host quality, this may lead to competition between larvae within the same host, resulting in increased mortality (Withers, Madie & Harris 1997; Hoffmeister & Rohlfs 2001; Pexton *et al.* 2009) and/or decreased offspring fitness (Hardy, Griffiths & Godfray 1992; Withers, Madie & Harris 1997; Desouhant *et al.* 2000; Zaviezo & Mills 2000). In gregarious species, low-quality hosts have been reported to yield fewer offspring (Zaviezo & Mills 2000; West, Flanagan & Godfray 2001; Bezemer & Mills 2003; Peters 2010; Goncalves *et al.* 2012) and two mechanisms are likely to create this pattern in final offspring eclosion, namely the mother's initial clutch size decision or increased developmental mortality of the offspring.

To avoid severe competition and the resulting mortality and/or reduction in offspring fitness, parents may use information about quantity and quality of resources to produce an optimal clutch size. Optimal clutch sizes have first been studied in birds (Lack 1947); the so-called 'Lack Clutch Size' maximizes the number of offspring surviving to independence. Since then models of optimal clutch size have been extended including the state dependence of clutch size decisions (Mangel 1987) and the fitness of resulting offspring rather than mere survival to adulthood (e.g., Mangel, Rosenheim & Adler 1994). This theory has also been applied to insect parasitoids (Charnov & Skinner 1984), where the resources available to the offspring are likely to differ due to a host's size and/or health status. As a consequence, optimal clutch sizes are expected to vary with host quality (Skinner 1985), with low-quality hosts receiving smaller initial numbers of eggs and therefore harboring fewer offspring.

The aim of our study was to disentangle the respective contribution of offspring developmental mortality vs. mother's clutch size decision as determinants of offspring production in the parasitic wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). This small wasp (1.0–3.5 mm) is a gregarious parasitoid attacking a broad range of cyclorraphous Diptera (Whiting 1967). Previous studies have revealed that *N. vitripennis* offspring numbers differ on hosts of different qualities, such as previously parasitized hosts varying in age (King & Rafai 1970; Werren 1984) and number (Werren 1984; Shuker *et al.* 2005) of conspecific larvae already present in the host, on different host species (Smith 1969; Rivers & Denlinger 1995a; Rivers 1996), and on hosts of varying age (Wylie 1963; Rivers & Denlinger 1995a) or size (Wylie 1967; Rivers & Denlinger 1995a). Apart from Wylie (1967), who tested mortality on different sizes of hosts (*Musca domestica* L.), these studies were assessing clutch size based on either counts of eggs in dissected hosts or numbers of offspring hatched from pupae, but not both. Therefore, the question to which extend offspring production on hosts of different qualities depends on a female's clutch size decision or on developmental mortality has not been answered.

In our study, we investigated adaptive decision making by females of *N. vitripennis* determining the respective contribution of developmental mortality of offspring vs. clutch size decision of the mother on hosts of different qualities. We further examined the size of the resulting offspring as a proxy for the fitness consequences of these decisions. Our study examines how changes in host quality led to changes in clutch size decisions of females, hence what information is actually used by females, and how their decisions influence offspring fitness. The role of information use and its possible limits are of major interest to understand how organisms adjust their behavior in an adaptive way (Krebs & Davies 1997; Dukas 1998a).

3.3 Materials and methods

Study organisms

We used the *N. vitripennis* laboratory strain HVRx, a mixture of five lines collected in 2001 in the Hoge Veluwe area in The Netherlands (52°5′ N 5°48′ E) and cultured to maintain high genetic variability (BA Pannebakker & L van de Zande, pers. comm.). Wasps were kept in an incubator at 25 °C, 60% r.h., and L16:D8 photoperiod, they were reared on pupae of *Calliphora vomitoria* (L.) (Diptera: Calliphoridae) flies. Hosts were obtained as maggots from a local pet shop, kept at 25 °C in saw dust until 2 days after pupation, and transferred to 4 °C until use.

Figure 3.1 Wild type (**top**) and red-eyed mutant (**bottom**) female

To determine the age of the wasps used for the experiments and avoid a possible influence of competition experience, female wasps were sorted as pupae and kept individually in gelatine capsules (0.37 cm³) until hatching. Female pupae of *N. vitripennis* are easy to distinguish from males because the ovipositor is visible (Werren & Loehlin 2009c). Freshly emerged females were kept singly in polystyrene vials (27 × 60 mm) and fed 10 % (vol/wt) sugar solution. On the 1st day, every female was kept with a male to allow mating. Two days and 1 day before the experiment females were given access to one host for 4 h to allow host feeding for egg maturation. Female wasps were used for experiments 4–6 days after hatching. To be able to distinguish clutch sizes laid by HVRx females under competitive situations (i.e., previously parasitized hosts), we used females of a red-eyed mutant strain (STDR) that were kept under equivalent conditions (Figure 3.1).

Experiments

We created seven levels of host quality (plus a control, see below) with hosts varying in weight, age, and health status (Table 3.1). Hosts (*C. vomitoria*) were weighed on the day of pupation. Three host weight categories were used for the experiments: 52.5–57.5 mg (small), 67.5–72.5 mg (medium), and 82.5–87.5 mg (large). After weighing host pupae were kept at 25 °C for 2 days and then transferred to 4 °C until use if not indicated otherwise. To create preparasitized hosts, *N. vitripennis* females of the red-eyed mutant strain (STDR) were used for the first oviposition. This allows distinguishing offspring from the first and the second parasitoid female after eclosion. To ensure that mortality in preparasitized hosts does not result from mortality in the STDR strain, hosts parasitized solely by STDR females were used as a control (Table 3.1).

Table 3.1 Treatment of *Calliphora vomitoria* pupae for creating hosts of different quality

Host quality	Treatment of hosts
Small	52.5–57.5 mg, kept at 25 °C for 2 days
Medium	67.5–72.5 mg, kept at 25 °C for 2 days
Large	82.5–87.5 mg, kept at 25 °C for 2 days
Old	Medium host kept at 25 °C for 2 additional days
Dead	Frozen medium host thawed 24 h prior to experiment
STDR	67.5–72.5 mg, kept at 25 °C for only 1 day, parasitized by a red-eyed (STDR) female for 24 h
Preparasitized 1 day (p 1d)	STDR host, immediately used in experiment
Preparasitized 4 days (p 4d)	STDR host, kept at 25 °C for three additional days prior to experiment

To simplify egg counting, hosts were buried to two thirds in sand in small polystyrene vials (27 × 60 mm), such that only the anterior part of the pupa was accessible for the female. Partially burying the host does not influence host size estimation in *N. vitripennis* (Wylie 1967). To avoid self-superparasitism, females were given the opportunity to leave the host patch: vials were closed with a foam stopper that was pierced with a pipet tip, which led into a second, upper vial. Because females display negative geotaxis they could leave the host patch and would be prevented from going back into the test vial again. At the beginning of the experiment, females were released into the lower vial. They were then allowed to parasitize for a maximum of 24 h or until they left the test vial. Females were frozen immediately after testing. Their sizes (length of left hind tibia) and numbers of mature eggs inside their ovaries were determined later on. To estimate the numbers of mature eggs, females were dissected, their ovaries removed into a droplet of water, and eggs were immediately counted. Eggs were categorized as immature, mature, or resorbed by their size and shape according to criteria described by Edwards (1954a).

For each of the seven host qualities (plus the STDR control) 40 replicates were made, 20 of which were randomly chosen and dissected immediately after parasitization to count the eggs and young larvae present (early dissection), whereas the remaining 20 were kept at 25 °C for 15 days before the offspring was counted. At this time, most offspring had already emerged as mature wasps form the host pupae. Nevertheless, every host pupa was carefully opened to check for dead, not fully developed, or diapausing offspring of *N. vitripennis* (late dissection). Among the latter two developmental states, diapause larvae are easily recognized by their white fat cells visible through the cuticle (Werren & Loehlin 2009b). The size of the fully developed offspring was estimated by measuring the length of the left hind tibia, with the arithmetic mean of tibia length for all female offspring from one host being used in the statistical analysis. In *N. vitripennis* tibia length is correlated with female lifetime fecundity (Sykes *et al.* 2007) and is therefore a suitable proxy for offspring fitness.

Unexpected host mortality

When counting eggs and young larvae on the freshly parasitized pupae, all hosts appeared to be perfectly healthy. However, several of them had not been parasitized at all. When dissecting host puparia 15 days later, we noticed that some of them had become hollow by then and no traces of parasitoid offspring were detectable. Even though we assume that parasitoid females have been able to sense the unsuitability of these hosts and refrained from laying eggs on them, we do not know if this was the reason for refraining from egg laying in all non-parasitized hosts. Hence, all 'zeros' were kept in the data set and we dealt with the unexpected host mortality using zero-inflated negative-binomial models (ZINB; Zuur *et al.* 2009).

Statistical analysis

All analyses were performed with R 2.15.2 (R Core Team 2012) and package *car* (Fox & Weisberg 2011) using Generalized Linear Models (GLM; Nelder & Wedderburn 1972). The respective error distributions are given with the test results. For the zero-inflated negative-binomial (ZINB) models we used package *pscl* (Zeileis, Kleiber & Jackman 2008) and for subsequent selection of the minimal adequate model *lmtest* (Zeileis & Hothorn 2002). Graphs were made using packages *gplots* (Warnes 2012), *sfsmics* (Maechler & al. 2012), and *calibrate* (Graffelman 2012).

We tested for early developmental mortality by determining the number of offspring present immediately after the parasitization period of the focal female (early dissection) in half of the replicates and comparing it with the other half of the replicates, for which offspring determination was done 15 days later (late dissection), the latter including fully developed wasps, diapausing offspring, and dead/alive larvae/pupae. Thus early developmental mortality includes only egg and early larval stage mortality, where we posit that no traces of these early dying individuals can be found during late dissection (after 15 days). In two treatments, the hosts given to the focal female (strain HVRx) had been parasitized before by a red-eyed mutant female (strain STDR). As eggs and larvae of HVRx and STDR females cannot be distinguished, we used total offspring count in our comparison of developmental mortality instead of HVRx offspring count. This assumes that no developmental mortality occurs in STDR offspring and was thus checked experimentally: there were no significant differences in offspring counts between early (eggs and small larvae) and late (fully developed wasps, diapausing offspring, dead/alive larvae/pupae) dissected hosts (ZINB: $\chi2 = 0.1979$, d.f. = 1, $P = 0.66$; $n = 40$) within the STDR hosts (Table 3.1). Thus, no early developmental mortality occurred in STDR offspring. For late developmental mortality in STDR offspring we compared the proportion of fully developed offspring in STDR hosts (only parasitized by a red-eyed mutant strain female) to the proportion of fully developed offspring in medium hosts (only parasitized by a wild-type female). We found no significant differences in the proportion of fully developed offspring between red-eyed mutant (STDR) and wild-type (HVRx) offspring (GLM with quasibinomial distribution: $F_{1,23} = 2.2816$, $P = 0.14$; $n = 25$), thus no differences in late developmental mortality between red-eyed mutant and wild-type offspring.

Within the group of late dissected host puparia, the sex, size, and origin (HVRx or STDR mother) of not fully developed parasitoid offspring could not be determined, therefore clutches that contained more than 10 % of these offspring were excluded from clutch size decision and offspring size analysis ($n = 16$). The following dataset allowed for analysis of clutch size decisions of focal females (HVRx strain females only). For host qualities small, medium, large, and old hosts all available clutch size estimates were used (eggs, small larvae, and adult wasps). For dead hosts, only clutch sizes determined immediately after parasitization were used (eggs, small larvae), because of the high mortality during development. For previously parasitized hosts, only emerged adult HVRx offspring was counted; hosts that contained no red-eyed offspring, thus that were not previously parasitized, were excluded ($n = 13$). Egg load (number of mature eggs inside a female's ovaries plus the number of eggs laid during the experiment) was log-transformed and included as an explanatory variable in the clutch size decision analysis. For technical reasons, the size and egg load of females could not be determined in 15 out of 191 replicates, which were thus excluded from the clutch size decision analysis.

3.4 Results

Early developmental mortality

To determine early developmental mortality, we compared numbers of offspring present in host pupae dissected either immediately after parasitization (eggs and small larvae) or 15 days later (fully developed wasps, diapausing offspring, dead/alive larvae/pupae). The full ZINB model used contained host quality and dissecting time and the respective interaction term as explanatory variables. The interaction between dissecting time and host quality within the count model was significant (χ^2 = 12.826, d.f. = 6, P = 0.046; n = 280). We therefore analyzed the data for each of the seven host qualities (Table 3.2) separately with dissecting time as explanatory variable.

Table 3.2 Comparisons of *Nasonia vitripennis* offspring counts on early and late dissected hosts of different host quality. Statistical data represent the output of ZINB models (d.f. = 1, n = 40)

Host quality	χ^2	P
Dead	8.3638	0.0038
P 1d	0.9485	0.33
P 4d	0.0974	0.76
Old	0.2172	0.64
Small	1.5554	0.21
Medium	0.1283	0.72
Large	0.0199	0.89

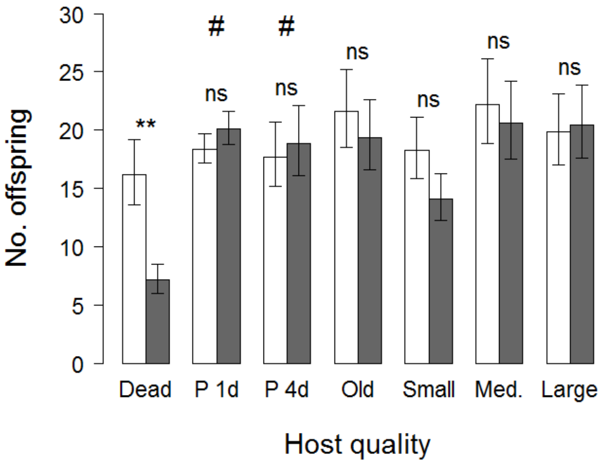

Figure 3.2 Mean (± SE) number of *Nasonia vitripennis* offspring counts on early (white) and late (gray) dissected hosts of different qualities. Dead hosts show significantly fewer old (fully developed wasps, diapausing offspring, dead/alive larvae/pupae) than young (eggs and small larvae) offspring ($P < 0.01$), indicating considerable mortality. In all other host qualities, differences between numbers of young and old offspring are insignificant.
Abbreviations: P 1d and P 4d: hosts preparasitized by STDR females 1 day and 4 days earlier, respectively; Med.: medium sized hosts; #: compound measurement of offspring from red-eyed mutant (STDR) and wild-type (HVRx) wasps. ns: $P > 0.05$, **$P < 0.01$.

A clear difference was only found in dead hosts, which contained significantly fewer old offspring (fully developed wasps, diapausing offspring, dead/alive larvae/pupae) than young offspring (eggs or small larvae) (Table 3.2). Early developmental mortality was approximately 56 % in this treatment (Figure 3.2). In all other host qualities, differences between early and late dissected host puparia (Figure 3.2) revealed no significant influence of dissecting time (Table 3.2), thus no significant early developmental mortality had occurred.

Late developmental mortality and diapausing larvae

When dissecting host puparia 15 days after parasitization, we noticed that not all offspring had completed development by then. Hosts of every quality contained large dead larvae (late developmental mortality), pupae (decelerated development), and large healthy larvae (diapausing larvae) to various degrees (Figure 3.3). The probability for completing development within 15 days was not influenced by host quality as the proportion of fully developed individuals did not differ between treatments (GLM with quasi-binomial distribution: $F_{6,82} = 1.7089$, $P = 0.13$; $n = 89$). The average proportion of fully developed offspring (adult wasps) was around 91 %.

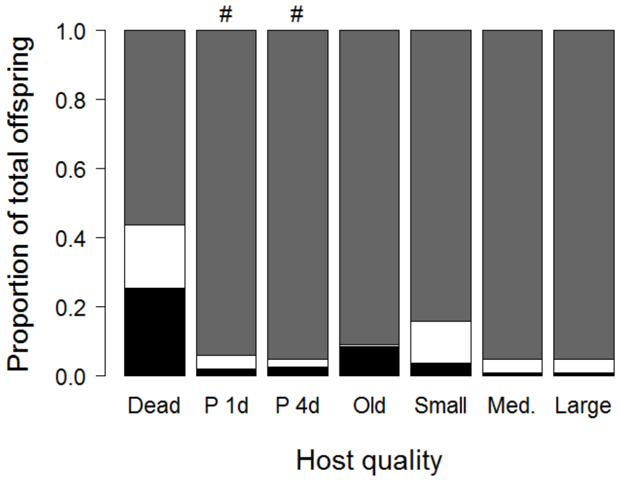

Figure 3.3 Fate of late developmental stages of *Nasonia vitripennis*: respective proportions of dead individuals (black), pupae/diapause larvae (white), and fully developed individuals (gray) within the various host qualities. Proportions of fully developed individuals did not differ between host qualities. Abbreviations: P 1d and P 4d: hosts preparasitized by STDR females 1 day and 4 days earlier, respectively; Med.: medium sized hosts; #: compound measurement of offspring from red-eyed mutant (STDR) and wild-type (HVRx) wasps.

Host quality dependent clutch size decisions and their fitness consequences

Host quality had a significant effect on clutch size, determined as number of eggs/small larvae or adult wasps, of the focal wild-type female (ZINB: $\chi^2 = 37.318$, d.f. = 6, $P < 0.001$; n = 176): focal wild-type females produced no offspring on hosts parasitized 4 days earlier, few offspring on hosts parasitized 1 day before, and a few more on small and dead hosts. Large, medium, and old hosts received the largest clutches from the focal female (Figure 3.4). Besides this, females laid bigger clutches at higher egg loads and this effect was more pronounced in small females compared to large ones (ZINB; tibia length * ln(egg load): $\chi^2 = 4.7402$, d.f. = 1, P = 0.029; n = 176). Within this analysis, two of the females that produced hatched offspring had only male offspring and might therefore not have been mated. Excluding these two data points did not influence the general outcome of the analysis, but only slightly changed P-values (ZINB; host quality: $\chi^2 = 30.68$, d.f = 6, $P < 0.001$; n = 174; tibia length * ln(egg load): $\chi^2 = 3.9886$, d.f. = 1, P = 0.046).

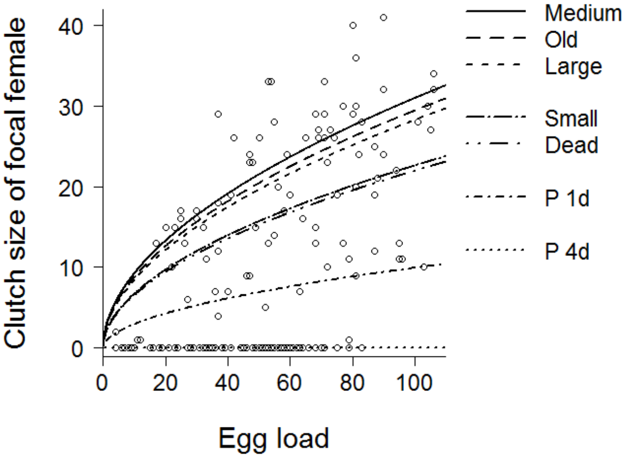

Figure 3.4 Numbers of eggs laid by the focal (HVRx) *Nasonia vitripennis* female on hosts of various qualities as a function of the egg load of the female, raw data (open circles) and regression lines for females with an average tibia length (0.642 mm) derived from the model.
Abbreviations: P 1d and P 4d: hosts preparasitized by STDR females 1 day and 4 days earlier, respectively.

Interestingly, female offspring fitness measures were not influenced by host quality (Figure 3.5A). Length of the left hind tibia of female offspring was used as a proxy for offspring fitness. As these data were left skewed, exp-transformed tibia length was used for the analysis to achieve a normal distribution of errors. An analysis of the exp-transformed mean length of the left hind tibia of female offspring revealed no significant differences between host qualities (GLM with Gaussian distribution: $F_{5,35}$ = 1.6091, P = 0.18; n = 41). In contrast, we found a slight effect on sex ratios (Figure 3.5B), based on AIC model optimization, although the overall model was not significant (binomial GLM: $F_{5,35}$ = 1.2067, P = 0.33; n = 43). Sex ratios in hosts previously parasitized by STDR wasps and dead hosts were less female biased than in old hosts.

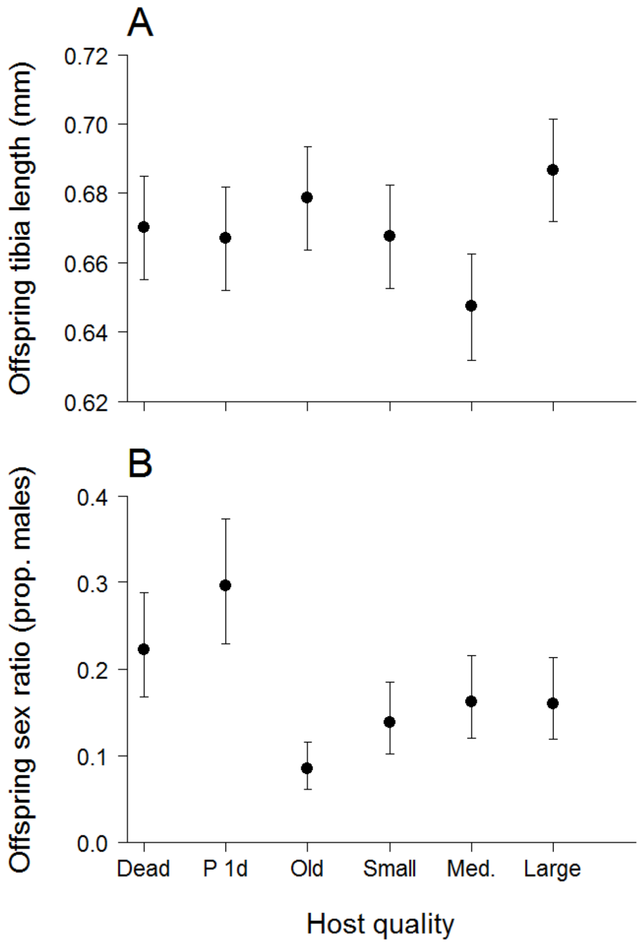

Figure 3.5 **(A)** Average female offspring size per clutch of *Nasonia vitripennis*. Mean (± SE) tibia length (mm) of female offspring hatched from hosts of various qualities. The relatively low value for offspring from hosts of medium size seems to reflect overall variability, as differences in offspring size between host qualities have not been significant in the statistical analysis. **(B)** Sex ratios of offspring produced on hosts of various qualities. Slight effects of host quality on sex ratio are suggested by AIC model optimization.

Abbreviations: P 1d: hosts preparasitized by STDR females 1 day earlier; Med.: medium sized hosts.

3.5 Discussion

Low-quality hosts have repeatedly been shown to yield lower offspring numbers for parasitoid insects (Zaviezo & Mills 2000; West, Flanagan & Godfray 2001; Bezemer & Mills 2003; Peters 2010; Goncalves *et al.* 2012)and two mechanisms may lead to this pattern: the clutch size decision made by the mother and developmental mortality among the offspring.

Early developmental mortality

In our data, negligible early developmental mortality on live hosts supports the hypothesis that differential offspring production results from adaptive decision making of females in *N. vitripennis*. Nevertheless, we detected high early developmental mortality (approximately 56 %) of eggs laid in already dead hosts.

Low early developmental mortality in *N. vitripennis* on all qualities of live hosts might be explained by a combination of high parental investment and a relatively stable and protected environment for the development of the larvae: they are sheltered by the host-surrounding fly puparium while eggs are deposited on the surface of the pupa, which places them out of reach of the host's immune system. Egg encapsulation for example is a major cause of mortality in endoparasitoid species (Schmidt, Theopold & Strand 2001; Kapranas *et al.* 2011). Additionally, females of *N. vitripennis* inject venom when stinging the host (Ratcliffe & King 1967), which seems to be a maternal investment into offspring development. Envenomation alters the host's metabolism and enhances developmental success of the larvae within this host (Rivers & Denlinger 1995b). It arrests host development and eventually kills it (Rivers & Denlinger 1994a). The venom alters the host's lipid metabolism such that there are more lipids within envenomated hosts compared to healthy hosts, which has been shown by Rivers & Denlinger (1994b) for *Sarcophaga bullata* (Parker). It also contains antimicrobial peptides (Ye *et al.* 2010) that may protect larval resources.

Dead hosts may represent a poor-quality environment for the development of the larvae because the venom cannot induce changes in metabolism (Rivers & Denlinger 1995b) and the nutrients in dead hosts might already be degraded. Without the potential immune stimulation by envenomation, *N. vitripennis* offspring may have to compete with microbes for host nutrients or even be at risk of becoming infected themselves (Danneels, Rivers & De Graaf 2010). In conclusion, there were no differences between the host qualities with respect to early developmental mortality, with the exception of dead hosts.

Late developmental mortality and diapausing larvae

Mortality due to resource shortage might not act on the early larval stages but on later stages, at times when most of the host tissue is already consumed. However, no significant differences in the proportion of fully developed individuals could be found between host qualities (Figure 3.3), although there seems to be a smaller proportion within dead hosts. In all host qualities, an average of 91 % of the detected offspring survived and accomplished development within 15 days. We thus conclude that even at later stages there is no severe competition between offspring that causes mortality and could thereby lead to reduced offspring numbers in low quality hosts.

Host quality dependent clutch size decisions

By ruling out developmental mortality as a cause for differential offspring production on live hosts of different qualities, only female decision making remains to explain the observed variation in offspring production. Females indeed modified their clutch size in response to host state, reducing it on smaller and parasitized hosts (including information about the age of the larvae already present) as well as on dead hosts. Therefore, females are able to sense the status of a host and to use this information for making adaptive clutch size decisions.

In contrast to a previous study by Wylie (1963) using *M. domestica* as host species, our data show no influence of host age on clutch size decisions of females (see Figure 3.4, regression lines ‘old’ vs. ‘medium’ hosts, P = 0.74) or offspring survival probability (see Figures 3.2 and 3.3, ‘old’ vs. ‘medium’ hosts). This is probably due to a host species effect. In *C. vomitoria*, the host’s age range (2–4 days) covered in our study is apparently suitable for parasitization by *N. vitripennis* without affecting offspring fitness.

Females decreased their clutch size in response to small hosts, which shows their ability to use host size as cue in their decision making process. Yet, how they measure host size is still an open question. *Nasonia vitripennis*, unlike, e.g., *Trichogramma minutum* Riley (Schmidt & Smith 1985), does not use surface area to estimate host size (Wylie 1967).

Surprisingly, no clutch size adjustments between medium and large hosts occurred (15 mg and 21 % weight difference); differences were only found in comparison to small hosts (15 mg and 27 % weight difference). The failure of females to respond to the differences between medium and large hosts suggests that size estimation in *N. vitripennis* is a non-linear process.

When superparasitizing, females adaptively reduced their clutch size in response to the numbers and state of the competitors of their offspring, refraining from oviposition in hosts (Figure 3.4), in which most of the host tissue of hosts parasitized 4 days prior to exposure had already been consumed and only large wasp larvae were present within the puparium. This is in line with previous studies (King & Rafai 1970; Shuker, Pen & West 2006). In contrast, hosts parasitized 1 day prior to the experiment containing only wasp eggs were provided with a few eggs (Figure 3.4) leading to offspring development without any apparent disadvantages in survival (Figures 3.2 and 3.3) or size (Figure 3.5A).

Interestingly, on dead hosts, female clutch size decision and offspring mortality have acted in concert in determining final offspring production. Reduction of clutch sizes in comparison with healthy hosts of comparable size (Figure 3.4) still has led to very high mortality occurring during early development of the offspring (Figure 3.2); yet, the size of surviving offspring from dead hosts did not differ from size of offspring from live hosts of the same weight (Figure 3.5A).

Most importantly, offspring size and thus offspring fitness measures did not differ among host qualities (Figure 3.5A), suggesting that clutch size decisions of females were well adapted to the quality of the hosts. Sykes et al. (2007) could show that an artificially increased clutch size and therefore a decrease in resource per offspring leads to decreased offspring size in *N. vitripennis*. This indicates that in our experiment females adjusted clutch sizes to keep the level of competition approximately the same, such that the fitness of resulting offspring did not differ among host qualities. This is remarkable considering the fact that sex ratio adjustments have been made by females on previously parasitized hosts and males require fewer resources because they are smaller than females (Whiting 1967).

Effects of female status on clutch size decisions

Besides the impact of external cues related to host quality, clutch size decisions in *N. vitripennis* were also influenced by a female's current egg load and her size, as suggested by state-dependent theory on optimal clutch size decisions in insects (Iwasa, Suzuki & Matsuda 1984; Mangel 1987). Interestingly, female size even affected the way egg load triggered clutch size decisions, suggesting that both egg load and abdominal volume for carrying mature eggs have to be taken into consideration to understand optimal clutch size decisions (see Mangel 1987) in organisms that mature eggs over their lifetime and produce eggs that are relatively large in relation to their abdominal size. In our experiment, the number of eggs laid on a given host increased with the number of mature eggs that were available to the female in a curvilinear function with monotonically decreasing slope (Figure 3.4). When thus oviposition opportunities exceed the number of eggs a female has available, she should reduce clutch sizes and thereby increase the fitness gained per egg, according to theory (Iwasa, Suzuki & Matsuda 1984). Thus, in addition to cues related to host status we have found *Nasonia* females to integrate information about their own status when deciding on how many eggs to lay. This corresponds with findings for other insects with a parasitoid reproductive mode (sensu Price 1980), in which oviposition decisions are influenced by female age (Elzinga, Harvey & Biere 2005; Xu *et al.* 2012) or egg load (Rosenheim & Rosen 1991; van Randen & Roitberg 1996; Migani, Ekesi & Hoffmeister 2014).

In conclusion, our data clearly show that in line with state-dependent optimal foraging theory, for *N. vitripennis* state-dependent female clutch size decisions are the most important factor influencing the number of offspring produced per host, whereas offspring mortality is negligible in live *C. vomitoria* hosts.

3.6 Acknowledgments

We thank BA Pannebakker for sharing the HVRx and STDR strain with us, L Rogge for the genetic identification of the fly species used in the experiment, and two anonymous reviewers for helpful comments. This study was funded by the Central Research Development Fund, University of Bremen, grant 02/125/10 to AT.

4 Causes of intraspecific variability in associative learning in the parasitic wasp in *Nasonia vitripennis*

Mareike Koppik, Thomas S. Hoffmeister, Sven Brunkhorst, Melanie Kieß & Andra Thiel*

Institute of Ecology, University of Bremen, Leobener Straße, 28359 Bremen, Germany

*Andra Thiel, E-mail: thiel@uni-bremen.de, Telephone: +49 (0)421 218-62937, Fax: +49 (0)421 218-62949

Keywords: host finding, memory formation, parasitoids, cue reliability, classical conditioning, intraspecific variation

In preparation for resubmission to *Animal Cognition*

4.1 Abstract

Within the animal kingdom the ability to learn is key to behavioral adaptation to changing environments. Yet, learning behavior varies cross species. As a prerequisite for the evolution of species-specific or even population-specific learning behavior considerable genetic and phenotypic variability within that trait needs to be present within species. While several species of parasitoid wasps have been shown to have the ability to learn associating chemical cues with the presence of hosts, intraspecific variability in this learning behavior has received little attention. Here we show that learning of host associated cues in the parasitic wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) shows considerable variability, which is at least partly genetic and also varies with cue reliability. We tested memory retention in a genetically diverse strain and in an iso-female line, bearing a low genetic variability. Our findings suggest that the two strains differ in memory formation from each other and also from yet another strain of *N. vitripennis* used in previous studies. The genetically diverse strain in our study showed much more variability in memory retention than did the iso-female line, and this variability decreased with increasing cue reliability. Even though the associative learning of host cues is most likely under strong natural selection in parasitoid females, genetic variability is maintained within the species.

4.2 Introduction

The ability to learn is an almost ubiquitous trait in the animal kingdom. It has been shown to be advantageous for an individual in many contexts (Papaj & Vet 1990; Danci *et al.* 2013). However, there are various direct and indirect costs associated with learning behavior (e.g. Mery & Kawecki 2004; Mery & Kawecki 2005; Burger *et al.* 2008; Snell-Rood, Davidowitz & Papaj 2011). These costs have an influence on e.g. what should be learned, when learning should occur and how fast an individual should learn (Eliassen *et al.* 2009). Therefore, it is not surprising that studies on animal cognition have revealed high variation in cognitive abilities (Healy *et al.* 2009), despite the rather high consistency in molecular mechanisms of learning and memory formation throughout the animal kingdom (Dubnau 2003).

There are several levels at which the variability in learning behavior can occur: between and within species (and between and within populations at the species level). Differences between species have led to investigations on ecological causes of species-specific learning behavior (Healy & Jones 2002). Differences within a species have been linked to differences in ecology between populations (Thiel, Schlake & Kosior 2013), to various trade-offs with life history parameters (Burger *et al.* 2008; Kolss & Kawecki 2008; Snell-Rood, Davidowitz & Papaj 2011) or even to individual differences throughout life like nutritional status or age (Tamura *et al.* 2003; Placais & Preat 2013).

Promising model systems in the study of species-specific learning behavior and its underlying ecological causes are parasitoids, because closely related species often have very different ecologies (Hoedjes *et al.* 2011). Many parasitoid females show the ability of associative learning of host related chemical cues (Kaiser *et al.* 2003; Takasu & Lewis 2003; Bleeker *et al.* 2006; Muller *et al.* 2006; Smid *et al.* 2007; Schurmann *et al.* 2009; van den Berg *et al.* 2011; Hoedjes *et al.* 2012; Kruidhof *et al.* 2012; Schurmann *et al.* 2012; Thiel, Schlake & Kosior 2013; Hoedjes & Smid 2014). This behavior facilitates host finding and therefore increases a female's reproductive success and in turn its fitness (Papaj & Vet 1990). Two frequently studied parasitoid species are the closely related wasp species *Cotesia glomerata* and *Cotesia rubecula* (Hymenoptera: Braconidae), both parasitizing caterpillars of pierid butterflies. Corresponding to the various differences in their oviposition behavior and in the ecology of their hosts these two parasitoids also show considerable differences in their learning and memory retention behavior (Bleeker *et al.* 2006). Attempts have been made to link these differences also to genetic differences between the two species (van den Berg *et al.* 2010). Another example of interspecific differences in learning behavior in parasitoids are three closely related *Nasonia* species parasitizing pupae of various flies. A comparison of the learning abilities of *Nasonia vitripennis*, *Nasonia longicornis* and *Nasonia giraulti* revealed that all three species show different memory retention over time (Hoedjes *et al.* 2012). One explanation offered by the authors is the difference in host range of the three species. But the authors also point out that testing more populations of each species is necessary to clearly disentangle variability between and within species. A comparison of *N. vitripennis* and *N. giraulti* shows that both species can form long term memory but that *N. giraulti* needs spaced training to do so while *N. vitripennis* only needs one oviposition or host feeding event on the host (Hoedjes & Smid 2014). Our study focuses on one species within the genus *Nasonia*, namely *Nasonia vitripennis*, analyzing intraspecific variability of learning behavior within this species.

Intraspecific variability in learning behavior has been intensely studied in *Drosophila melanogaster* (Diptera: Drosophilidae) (Kawecki 2010), but has also received some attention in studies on parasitoid learning behavior. In *C. glomerata* different reward values (oviposition into a high or low value host) lead to different forms of memory (Kruidhof *et al.* 2012). Moreover, there is sufficient intraspecific variation within this species to shift learning through artificial selection to a slower learning rate, i.e. formation of long term memory requires several spaced training trials instead of a single trial (van den Berg *et al.* 2011). Different populations of the parasitic wasp *Venturia canescens* (Hymenoptera: Ichneumonidae) that differ in their reproductive mode and their natural habitat, show differences in their learning behavior (Thiel, Schlake & Kosior 2013); while thelytokous females from relatively homogeneous habitats quickly form memory that starts vanishing at 24 h after training, arrhenotokous females from more heterogeneous habitats show an increased memory retention after 24 h compared to earlier time points.

Despite the forces driving the evolution of species-specific learning ability, trade-offs between learning and other traits may sustain considerable (genetic) variation within one species. This genetic variation and the potential for evolution of better learning ability (i.e. fewer conditioning cycles needed for learning and slower decay of memory) has been demonstrated in a selection experiment in *D. melanogaster* (Mery & Kawecki 2002). Since then trade-offs between learning behavior and somatic maintenance have been intensely studied in *D. melanogaster* (Kawecki 2010). These studies could demonstrate indirect costs of learning, like a symmetric trade-off between longevity and learning ability (Burger *et al.* 2008) as well as a trade-off between learning ability and larval competitive ability (Mery & Kawecki 2003; Kolss & Kawecki 2008). Additionally, direct costs of learning and memory formation have been demonstrated. Formation of long term memory leads to an earlier death in *D. melanogaster* flies that have been food and water deprived after learning (Mery & Kawecki 2005). Moreover, repeated learning events have been shown to lead to a reduction in egg-laying rate (Mery & Kawecki 2004; Mery & Kawecki 2005). Costly memory forms like aversive long-term memory are disabled when *D. melanogaster* is deprived of food for approximately 24 h before training (Placais & Preat 2013). So, within the model species *D. melanogaster* there are several studies demonstrating the intraspecific variability in associative learning. But other species have been studied with respect to variability and costs of learning behavior, as well. The butterfly *P. rapae* (Lepidoptera: Pieridae) shows interfamily variation in learning ability and a constitutive trade-off between learning ability and fecundity (Snell-Rood, Davidowitz & Papaj 2011). On the other hand, in bumble bee colonies immune response and learning ability are positively correlated (Alghamdi *et al.* 2009).

D. melanogaster is a good model system for evolutionary experiments on learning because of short generation times (approx. 2 weeks) and the genetic tools available (Kawecki 2010). The genus *Nasonia* bears similar features (Werren *et al.* 2010) and may therefore be a good study system to confirm and extend knowledge about costs and benefits of learning and memory gathered in *Drosophila*. Two studies on associative learning of olfactory cues in *N. vitripennis* have revealed that different kinds of host experiences induce different kinds of memory. The first study demonstrated that a single drilling event into a suitable host pupa in the presence of an odor induces the formation of medium term memory (MTM) for that odor (Schurmann *et al.* 2009). A second study revealed that more intensive host experiences lead to the formation of a protein biosynthesis dependent long term memory (LTM) (Schurmann *et al.* 2012).

The aim of the present study was to investigate variability in learning within the parasitic wasp *N. vitripennis*. To exclude variation caused by the physiological state of a female we treated all females in the same way before and after conditioning and only used females in a very narrow age range (3–5 days). To test if variability is due to genetic differences or due to uncontrollable environmental factors during our training and testing procedure, we studied learning and memory in a highly genetically diverse *N. vitripennis* strain (HVRx, van de Zande *et al.* 2014) and in an iso-female line (AsymCX, Werren *et al.* 2010). To investigate the effect of cue-reliability on memory formation and variability we used conditioning treatments with increasing host experience intensity and increasing number of host encounters (increasing information gained and reliability). Our definition of memory forms follows a model of *N. vitripennis* memory dynamics proposed by a previous study done by Schurmann and colleagues (2012), with short term memory (STM) lasting up to one hour, early MTM lasting up to one day, late MTM being present 2 to 3 days after training and LTM lasting more than 3 days.

4.3 Material and methods

Study organisms

For the experiments two different laboratory strains of *Nasonia vitripennis* were used: HVRx and AsymCX. HVRx is a mixed strain from five different lines collected in 2001 in The Netherlands (52°1′ N 5°3′ E), cultured to maintain a high genetic variability (van de Zande *et al.* 2014). The rearing protocol established by van de Zande et al. that maintained a mean heterozygosity $H_E = 0.56 \pm 0.03$ over 32 generations (van de Zande *et al.* 2014) was followed to ensure genetic variability within the HVRx strain. AsymCX was obtained from an inbred laboratory strain and was further inbred through one generation of mother son crossing and at least 12 generations of sib matings, which should result in a very low genetic variability (Werren *et al.* 2010). Wasps were kept in an incubator at 25 °C and 60 % r.h. under a L16:D8 regime and reared on pupae of *Calliphora vomitoria* (L.) (Diptera: Calliphoridae) flies. Hosts were obtained from a local pet shop and kept at 25 °C in saw dust until two days after pupation. Afterwards they were transferred to 4 °C until use.

After hatching wasps were kept in groups in polystyrene vials (27 x 60 mm) and fed with 10 % (vol/wt) sugar solution until training. Female wasps were used in the training 3 to 5 days after hatching.

Conditioning

The conditioning was conducted at room temperature. Females were conditioned individually in petri dishes (diameter: 6 cm). Where applicable, the odor source was 50 mg (± 5 mg) of cinnamon presented in a small petri dish (diameter: 1 cm), the cinnamon source varied between experiments but was always the same for training and the subsequent testing. Where applicable (all conditioning treatments and control treatment 3, see Table 4.1), the hosts used for the conditioning were 2 day old *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) pupae (kept at 4 °C until use). *L. sericata* was used for conditioning because pupae are easier to drill into for inexperienced females than *C. vomitoria* pupae (personal observation). Additionally, *L. sericata* was used in previous studies (Schurmann *et al.* 2009; Schurmann *et al.* 2012) making our results better comparable to existing work. Hosts were placed singly (experiments 2 and 3) or in groups of three (experiment 1) in the petri dish for the conditioning of *N. vitripennis* females.

After the target behavior (one of a series of host encounter related behaviors conveying cues of different levels of information about hosts, given in Table 4.1) occurred females were gently brushed off the host/ out of the petri dish and kept singly with honey (experiment 1) or 10 % (vol/wt) sugar solution (experiments 2 and 3) as food until testing.

Table 4.1 Description of control (No. 1–3) and conditioning (No. 4–8) treatments used in the experiments (also see chapter 2.3)

No.	Treatment	Description	Experiment
1	Naïve	Females had no contact with hosts or odors (cinnamon and FFH) between hatching and testing	2, 3
2	Odor Control	Females were kept in a petri dish with an odor (cinnamon) for the time of training but had no access to a host	1, 2, 3
3	Host Control	Females were kept in a petri dish with one host but no odor until tapping (examination of the host surface with the tip of the abdomen) occurred	2, 3
4	Tapping	Females were kept in a petri dish with an odor and one and three hosts respectively until tapping (examination of the host surface with the tip of the abdomen) occurred	1, 2, 3
5	1 x Drilling	Females were kept in a petri dish with odor and one and three hosts respectively until end of drilling (successful insertion of ovipositor into the host puparium)	1, 3
6	2 x Drilling	First conditioning (see 1 x Drilling) was followed by a 2 h break and a second identical conditioning	1, 3
7	3 x Drilling	First conditioning (see 1 x Drilling) was followed by two cycles of a 2 h break and an additional identical conditioning	1
8	Host feeding	Females were kept in a petri dish with an odor and three hosts until the end of host feeding (intake of host's body fluids through a feeding tube built by the female), after a total of 1 hour host feeding was interrupted by the experimenter	1

Olfactometer tests

Memory retention was tested in a static four-chamber olfactometer, as has previously been used in experiments on associative learning in *N. vitripennis* (Schurmann *et al.* 2009; Schurmann *et al.* 2012). The cylindrical olfactometer consists of four chambers, in which odor sources can be placed, a walking arena (diameter: 20 cm) made of gauze that can be placed above the chambers and a glass plate to cover the walking arena and prevent the wasps from flying away during testing (for a detailed figure see Thiel, Schlake & Kosior 2013). In all tests 50 mg (± 5 mg) of cinnamon was placed in one chamber and a glass capillary (20 µl) approximately half filled with Furfuryl heptanoate (FFH, >= 98 %, SAFC®) was placed in the opposite chamber, with the two other chambers being left empty. To prevent females from orienting by visual cues, a filter paper was placed in every chamber to cover the contents. Females were released singly into the olfactometer out of a reaction tube that was placed in the center of the walking arena. The time females spent walking above each field after they left the tube was recorded for seven minutes with a stop watch (experiment 1) or the software 'The Observer XT' (Noldus, Wageningen, NL) (experiment 2 and 3). Females that did not leave the tube after 20 minutes were excluded from the analysis. After each test the glass plate and walking arena were replaced to avoid any effects of previous females (e.g. through odor traces). The olfactometers were used for up to four successive replicates, yet, after each trial they were rotated about 90° to avoid any effects caused by side preferences of female wasps.

Statistical Analysis

In the statistical analysis only walking times above the two chambers containing an odor source were included. The proportion of the time spent above the field containing cinnamon was analyzed with Generalized Linear Models (GLM, Nelder & Wedderburn 1972) with a binomial error distribution using the software R 3.0.2 (R Core Team 2013) and the package *car* (Fox & Weisberg 2011). Post-hoc multiple comparisons of means were done using the package *multcomp* (Hothorn, Bretz & Westfall 2008).

Graphs were prepared using R 3.0.2 (R Core Team 2013) and packages *gplots* (Warnes 2012), *calibrate* (Graffelman 2012) and *Hmisc* (Harrell, Dupont & al. 2013).

Control treatments

To exclude that changes in preferences for one or the other odor in our memory retention tests came from sensitization or habituation elicited by the odor or host alone, several controls were made for both the HVRx and AsymCX strains within experiment 2 and 3 respectively (Table 4.1). In those controls training procedure either contained only a tapping experience on the host (Host control), only an odor source (Odor control) or none of the two (Naïve).

Experiment 1

Similar to experiments that have previously been conducted with another strain of *Nasonia vitripennis* (Schurmann *et al.* 2009; Schurmann *et al.* 2012), several different behaviors (Table 4.1) were tested for their potential to elicit memory formation for an odor in females of the HVRx strain. Control and conditioning treatments lasted a maximum of 30 minutes (Odor control, Tapping, Drilling) or 60 minutes (Host feeding), females that did not show the target behavior after 30 minutes were excluded from the experiment. Females were tested 1 and 2 days (only Odor control and Tapping) and 3, 4, 5, 6 and 7 days after training (all treatments). For each time-treatment combination 14–26 replicates were made.

Experiment 2

In this experiment a finer time scaling of memory that was formed after a single tapping experience (Table 4.1) in the HVRx strain was analyzed. All treatments lasted a maximum of 10 minutes. Females that did not show the target behavior after 10 minutes were excluded from the experiment. Naïve and Host control females were tested 2 hours after treatment, all others were tested 10 minutes, 2 and 4 hours, 1, 2, 3, 4 and 5 days after treatment. For each time-treatment combination 15–29 replicates were made.

Experiment 3

To test memory acquisition in the AsymCX strain that has a very low genetic variability, three different conditioning and three control treatments were used (Table 4.1). Females that did not show the target behavior after 10 minutes (Host control, Tapping) or 30 minutes (1 x Drilling, 2 x Drilling) were excluded from the experiment. Naïve and Host control females were tested approximately 1 hour (45–90 minutes) after treatment, all others were tested 1 hour (45–90 minutes) and 3 and 5 days after treatment. For each time-treatment combination 20–22 replicates were made.

4.4 Results

Control treatments

To test whether an experience with only the cinnamon odor or only the host without an offered odor source already lead to a change in preference we compared females with those experiences to naïve females. In the HVRx strain naïve females, females that had an experience with the odor only (Odor control) and females that had an experience with a host only (Host control) did not differ significantly in their proportional walking time over cinnamon (GLM, binomial error distribution corrected for overdispersion, N = 58, treatment: $F_{2,55}$ = 2.8366, P = 0.067); overall HVRx females spent 48 % of their walking time over cinnamon. The marginally insignificant effect of treatment (P = 0.067) is caused by the slightly lowered proportional walking time on cinnamon in the Host control (Figure 4.1), at least suggesting that there is no sensitization effect of cinnamon. In the AsymCX strain naïve females, females that had an experience with the odor only (Odor control) and females that had an experience with the host only (Host control) did not differ significantly in their proportional walking time over cinnamon (GLM, binomial error distribution corrected for overdispersion, N = 60, treatment: $F_{2,57}$ = 1.0931, P = 0.342); overall AsymCX females spent 60 % of their walking time over cinnamon.

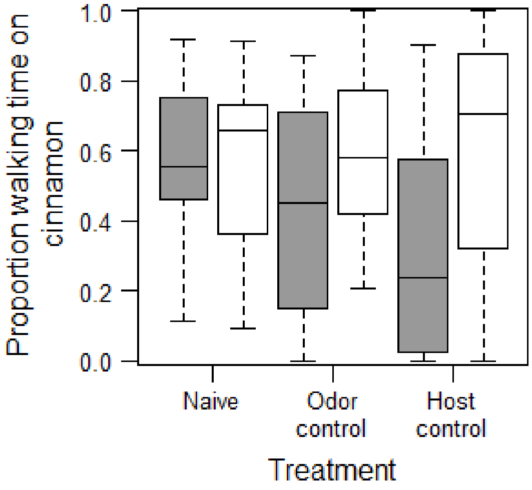

Figure 4.1 Variance in proportional walking time above the two odor fields for the three control treatments. Grey boxplots: HVRx strain, white boxplots: AsymCX strain. The thick line represents the median, the lower and upper end of the box represent the lower and upper quartile respectively and the whiskers extend to the most extreme points that are within a 1.5 * interquartile range

Moreover females from both strains and all three controls showed approximately the same variance in their proportional walking time on the cinnamon odor field (Figure 4.1, Table 4.2).

Table 4.2 Variance in arcsine square root transformed proportions of walking time on cinnamon; boxplots of corresponding untransformed raw data are shown in Figures 4.1 (a Controls), 4.3 (b Conditioning intensities) and 4.4 (c Time until testing). NA – not tested

		Variance	
	Treatment	HVRx strain	AsymCX strain
a Controls	Naïve	0.0678	0.0816
	Odor only	0.1184	0.0761
	Host only	0.1675	0.1801
b Conditioning intensity (testing: 3–7 days (HVRx) and 3 & 5 days (AsymCX) after conditioning)	**Treatment**		
	Tapping	0.1704	0.0949
	1 x Drilling	0.1238	0.0282
	Host Feeding	0.0982	NA
	2 x Drilling	0.1029	0.0102
	3 x Drilling	0.0536	NA
c Time until testing (across all conditioning treatments)	**Time**		
	1 hour	NA	0.0381
	3 days	0.1530	0.0600
	4 days	0.1224	NA
	5 days	0.1613	0.0599
	6 days	0.1313	NA
	7 days	0.1338	NA

Experiment 1

Within this experiment we investigated medium and long term memory formation of HVRx females after different experiences (treatment) on a host. The time span between treatment and testing did not influence the proportional walking time of females on the cinnamon odor field (GLM, binomial error distribution corrected for overdispersion, N = 539; time * treatment: $F_{5,527} = 0.3998$, $P = 0.849$, time: $F_{1,532} = 1.2162$, $P = 0.271$). But treatment had a significant influence on the proportional walking time (GLM, binomial error distribution corrected for overdispersion, N = 539, treatment: $F_{5,533} = 49.214$, $P < 0.001$). A tapping experience did not result in a significant increase in proportional walking time in comparison with control females (Odor control) (Figure 4.2, Table 4.3); these females do not show evidence of MTM or LTM formation for cinnamon. All other conditioning treatments led to a significantly higher proportion of time spent over the cinnamon field compared to control females (Odor control) (Figure 4.2, Table 4.3);

females with these experiences on a host seem to have formed an LTM (up to at least 7 days) for cinnamon.

Table 4.3 Multiple comparisons of mean proportional walking times of experiment 1 between various conditioning groups and the Odor control group, P values are adjusted with the Dunnett test for multiple comparisons

Conditioning	z	P
Tapping	1.189	0.681
1 x Drilling	5.523	< 0.001
Host feeding	8.379	< 0.001
2 x Drilling	8.371	< 0.001
3 x Drilling	11.329	< 0.001

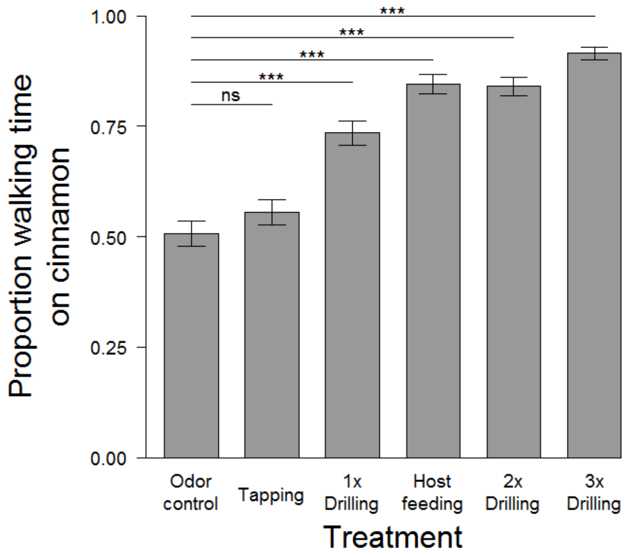

Figure 4.2 Experiment 1, estimates (± SE) of proportional walking time above the cinnamon field for different treatments. HVRx females were conditioned on cinnamon and tested on cinnamon versus FFH 1-7 days (Odor control, Tapping) or 3-7 days (other) after treatment. Time interval between treatment and testing had no significant influence on the proportion of time females spent walking over cinnamon and is not plotted here
ns: P > 0.05, *** P < 0.001

Variance in proportional walking time decreased with increasing conditioning intensity, being generally higher in the HVRx strain compared to the AsymCX strain (Figure 4.3, Table 4.2). No systematic change in variance can be observed with increasing time between conditioning and testing in the HVRx strain (Figure 4.4, Table 4.2).

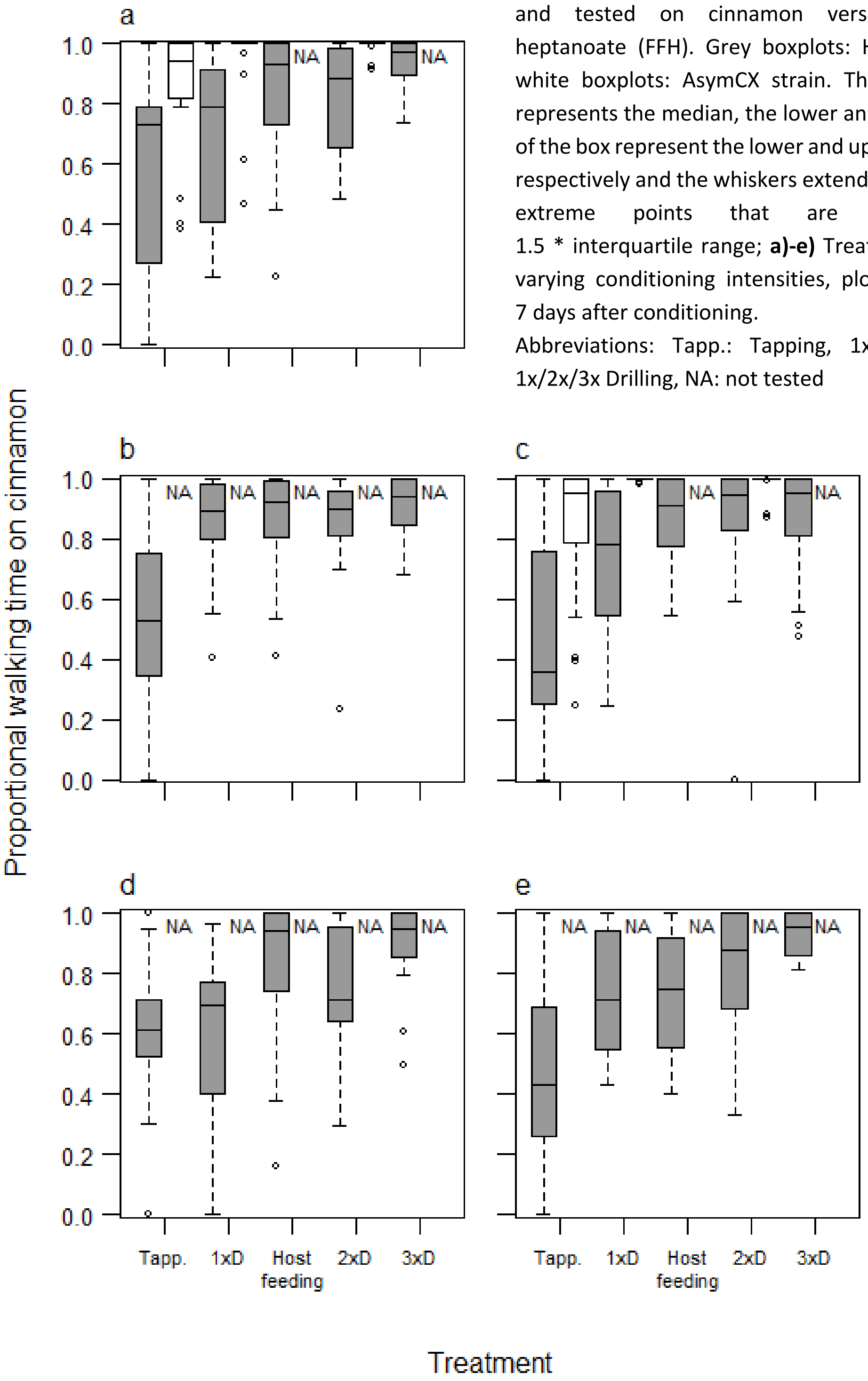

Figure 4.3 Variance across replicates of experiment 1 (HVRx) and 3 (AsymCX) respectively in proportional walking time above the two odor fields: females were conditioned on cinnamon and tested on cinnamon versus furfuryl heptanoate (FFH). Grey boxplots: HVRx strain, white boxplots: AsymCX strain. The thick line represents the median, the lower and upper end of the box represent the lower and upper quartile respectively and the whiskers extend to the most extreme points that are within a 1.5 * interquartile range; **a)-e)** Treatments with varying conditioning intensities, plotted for 3–7 days after conditioning.

Abbreviations: Tapp.: Tapping, 1xD/2xD/3xD: 1x/2x/3x Drilling, NA: not tested

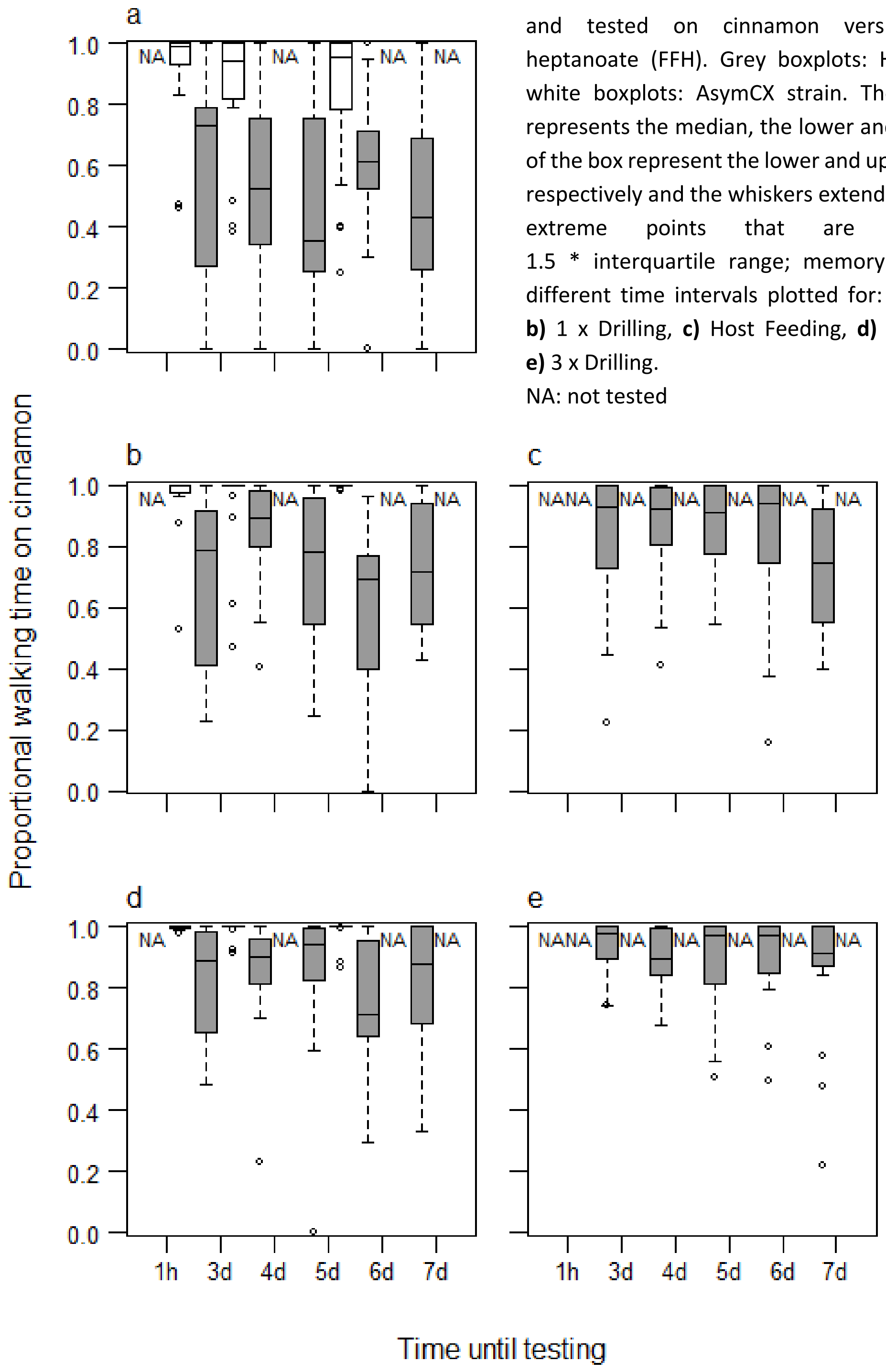

Figure 4.4 Variance across replicates of experiment 1 (HVRx) and 3 (AsymCX) respectively in proportional walking time above the two odor fields: females were conditioned on cinnamon and tested on cinnamon versus furfuryl heptanoate (FFH). Grey boxplots: HVRx strain, white boxplots: AsymCX strain. The thick line represents the median, the lower and upper end of the box represent the lower and upper quartile respectively and the whiskers extend to the most extreme points that are within a 1.5 * interquartile range; memory tests after different time intervals plotted for: **a)** Tapping, **b)** 1 x Drilling, **c)** Host Feeding, **d)** 2 x Drilling, **e)** 3 x Drilling.

NA: not tested

Experiment 2

The memory formation after a tapping event was investigated in the HVRx strain including short time intervals to test for STM and early MTM. Time had an influence on the proportional walking time of females and the effect was significantly different between the two treatments (GLM, binomial error distribution corrected for overdispersion, N = 400; time * treatment: $F_{1,396} = 29.251$, $P < 0.001$). While control females spent about 50 % of their walking time on cinnamon independent of the time between treatment and testing, females with a tapping experience spent a significantly higher proportion of their time on cinnamon between 10 minutes and 3 days after conditioning, slowly decreasing until they did not differ from control females anymore 4 and 5 days after conditioning (Figure 4.5). Therefore, we conclude that females have formed STM, early and late MTM but no LTM for cinnamon.

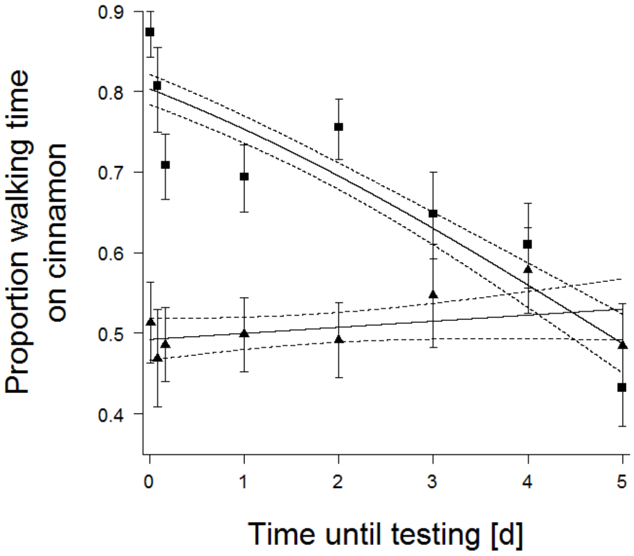

Figure 4.5 Experiment 2, proportional walking time of HVRx females above the cinnamon field as a function of time until testing. Wasps were conditioned on cinnamon and tested on cinnamon versus FFH 10 min to 5 days after training. Mean proportions and regression lines ± 1 * SE confidence interval for control group (Odor control, triangles, lower regression line) and trained group (Tapping, squares, upper regression line)

Experiment 3

To compare memory formation in the genetic diverse strain (HVRx) to that in an iso-female line (AsymCX) the influence of different experiences on memory retention was tested in the AsymCX strain. Time between treatment and testing did not influence the proportional walking time of females (GLM, binomial error distribution corrected for overdispersion, N = 246, time * treatment: $F_{6,234} = 1.0127$, $P = 0.418$, time: $F_{2,240} = 1.6445$, $P = 0.195$). But treatment had a significant influence on the proportional walking time

(GLM, binomial error distribution corrected for overdispersion, N = 246, treatment: $F_{3,242}$ = 71.392, $P < 0.001$). All conditioning treatments led to a higher proportion of time spent over the cinnamon field compared to the control treatment (Odor only), with Tapping leading to a slightly lower proportion than the other two conditioning treatments (Figure 4.6, Table 4.4). However, all conditioning treatments elicited the formation of LTM (up to at least 5 days) for cinnamon.

Table 4.4 Multiple comparisons of mean proportional walking times of experiment 3 between various conditioning groups and the Odor control group, P values are adjusted with the Dunnett test for multiple comparisons

Conditioning	**z**	**P**
Tapping	7.756	< 0.001
1 x Drilling	8.525	< 0.001
2 x Drilling	7.756	< 0.001

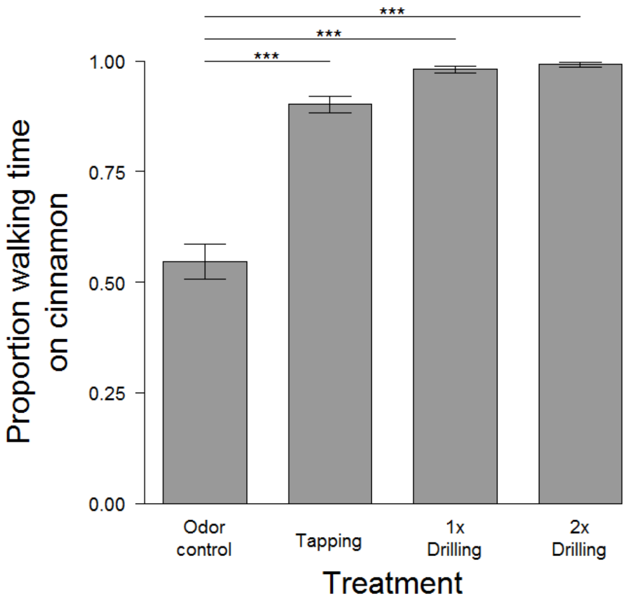

Figure 4.6 Experiment 3, estimates (± SE) of proportional walking time of AsymCX females above the cinnamon field for different treatments. Wasps were conditioned on cinnamon and tested on cinnamon versus FFH 1 hour and 3 and 5 days after treatment. Time interval between treatment and testing had no significant influence on the proportion of time females spent walking over cinnamon and is not plotted here
*** $P < 0.001$

Variance in proportional walking time decreased with increasing conditioning intensity, being generally lower in the AsymCX strain compared to the HVRx strain (Figure 4.3, Table 4.2). No systematic change can be observed with increasing time between conditioning and testing in the AsymCX strain (Figure 4.4, Table 4.2), though with a tapping experience an increase in variability with time can be observed (Figure 4.4a).

4.5 Discussion

After encountering a host in the presence of an odor, females of *Nasonia vitripennis* show an increased preference towards that odor when tested in an olfactometer. Our controls confirm that this increased preference is not evoked by the host encounter or the odor alone but only when odor and hosts are presented together. This leads to the conclusion that the increased preference is due to associative learning.

Previous experiments with a different strain of *N. vitripennis* (originating from the area of Hamburg, Germany) had shown that females form only an intermediate memory for an odor lasting up to 3 days after a single drilling experience on a host (Schurmann *et al.* 2009) and long term memory formation is restricted to a more intensive host experience (e.g. including oviposition or host feeding) (Schurmann *et al.* 2012). In contrast, our results show that after a single successful drilling event both strains used (HVRx and AsymCX) formed a longer lasting memory up to at least 5 (AsymCX, Figure 4.6) and 7 (HVRx, Figure 4.2) days respectively, which is most likely long term memory (Schurmann *et al.* 2012). Differences between the present study and the work by Schurmann and colleagues (Schurmann *et al.* 2009; Schurmann *et al.* 2012) might be caused by the different populations or by the slightly different experimental protocols used. Repeating part of the experiment with the HVRx strain and the setup used by Schurmann et al. (frozen host pupae for conditioning and olfactometer tests with cinnamon against empty chamber) in our lab still resulted in long term memory after a single drilling experience in the HVRx strain (see 4.7 Supplementary material). This indicates that there is considerable variability between populations of *N. vitripennis* in the memory formed after a certain behavior. In the AsymCX strain even a single tapping experience on the host was sufficient to elicit memory lasting at least 5 days (Figure 4.6). In contrast, we never found the formation of long term memory after tapping in the HVRx strain: within one experiment we found the complete lack of memory formation (Figure 4.2) and the formation of late MTM (lasting up to 3 days) within another experiment (Figure 4.5). These differences between our two experiments on the HVRx strain likely reflect the high variability within this strain. Our experiments show that there is variation between strains of the same species in the behavior that is needed to form a long term memory during associative learning (AsymCX: tapping; HVRx: drilling; 'Hamburg strain' (Schurmann et al. 2012): host feeding). Even though these differences are somewhat subtle, since all strains need only a single host encounter of different intensity, strains clearly differ with respect to the intensity and informative contents of cues needed to elicit memory formation (see below).

Besides the variability between strains of *N. vitripennis*, females within our genetically diverse strain (HVRx) show considerable variation in their memory retention (Figure 4.3 and 4.4, Table 4.2). This variability decreases with increasing training intensity (Figure 4.3, Table 4.2). This indicates that variation within tests is most likely not due to variation in e.g. motivational status (that should on average be the same between training procedures) but due to variation in memory retention in females. Variation in memory retention between females may originate from two sources: 1. Females may differ in their learning rate (i.e. training that is needed to trigger learning). 2. Females may differ in the length of memory that is formed after a certain training. If some females would form shorter memory than others (differences in memory length) we would expect an increase in variability over time. The fact that variation in memory retention does not systematically increase with time (Figure 4.4) suggests that females within the HVRx strain are rather consistent in the length of memory formed after training. Therefore, we conclude that females are more likely to differ in their learning rate, needing different numbers of trainings or training intensities to make an association with an odor.

In contrast to the genetically diverse strain the inbred strain of *N. vitripennis* (AsymCX) does show very low variability in memory retention (Figure 4.4a, 4.4b and 4.4d), while variability in walking behavior in control treatments is comparable to the HVRx strain (Figure 4.1). This confirms that variability in walking behavior found in the HVRx strain is not due to training or testing procedure, but most likely due to natural variability in learning behavior. Moreover, this result suggests that differences between females in their learning rate (i.e. training that is needed to trigger learning) have a strong genetic component, since reducing genetic variability also reduced variability in memory retention. This is in line with various other studies on insect learning that could show genetic variation in learning ability (see Dukas 2008).

Training intensity (i.e. number of trainings and/or duration of a single training event) should increase the reliability of the learned cue, since females can gather more information about the host and its quality and suitability the longer the host contact lasts. All behaviors used within this study (Table 4.1) usually occur during natural parasitization of a host by *N. vitripennis* in the following order: Tapping, Drilling and Host feeding. Females in the different training intensity groups only differed in the target behavior after which training was terminated by the experimenter. Tapping follows the first examination of a host, such that the female might have gained information about the size and probably about the host species encountered (due to chemical cues); additionally females might at this point detect drilling holes of preceding females thus gaining information about previous parasitization. A successful drilling into the host can provide the female with more information about the suitability of a host (e.g. health status, age, species and previous envenomation and parasitization of the host). Since females respond to cues inside a host puparium (King & Skinner 1991), they obviously sense such information with chemical receptors on the tip of their ovipositor after drilling as has been shown for other parasitoids (Quicke 1997, pp 189/190). Host feeding should also involve chemoreceptors, which might give additional information. This increase in information status provides females with increasing reliability with the information about an encounter with a suitable host. Besides more intense behaviors during one host encounter repeated encounters with a host associated with the same odor adds reliability to the learned cue. The more often a cue is encountered in the presence of a host the more likely it is that this cue reliably predicts the presence of hosts. In our experiment females differed in the training intensity and number of trainings that was necessary to trigger learning of an odor cue. We assume that females show a genetic difference in the cue reliability needed to trigger associative learning. This leads to the conclusion that within the HVRx strain there are females that quickly learn and therefore quickly adapt to changing environments but take the risk of learning an irrelevant cue, while there are others that need several training trials to make an association making them slow learners but at the same time putting them under low risk of learning irrelevant cues. Such variation might be important in a species like *N. vitripennis* that attacks hosts in diverse microhabitats from bird’s nests to animal carcasses (Abraham 1985; Voss, Spafford & Dadour 2009).

In conclusion we showed that there is high variability within the species *N. vitripennis* in their learning behavior even though there should be considerable selection pressure on that trait, since there is a close link between host finding success and fitness in parasitoids (Godfray 1994). Differences in the environment and hence slight differences in the ecology between populations can cause variability in learning behavior. Maintenance of variability in learning behavior might also suggest that there are trade-offs with e.g. life history traits. The close link between host finding success and fitness in parasitoids (Godfray 1994) and the high variability in learning of host associated cues we could show in our study, makes *N. vitripennis* an ideal system to study the costs and benefits of learning and memory. The sequenced genome and its comparatively short life cycle makes the *Nasonia* system comparable to the *Drosophila* system in many methodological aspects (Werren *et al.* 2010). On the other hand, the *Nasonia* system has its own specific features such as closely related interfertile species, the haplo-diploidy of hymenopteran species and the ecological adaptations going along with a parasitic life style (Werren *et al.* 2010). This opens up the possibility to study if phenomena found in *Drosophila* regarding the cost and benefits of learning also apply to other systems with very different ecologies. But the high variability between strains also shows that it is necessary to use several strains in comparative studies in order to clearly disentangle within and between species differences.

4.6 Acknowledgements

We thank H. M. Smid for sharing the AsymCX strain with us and L. Rogge for assistance with the olfactometer tests. We are grateful to two anonymous reviewers for helpful comments on the manuscript. This study was funded by the Central Research Development Fund, University of Bremen, grant 02/125/10 to AT.

4.7 Supplementary material

Influence of experimental protocol on memory retention in *Nasonia vitripennis*

Memory tests within this study have been conducted in a four chamber olfactometer with the odor females were trained on (cinnamon) in one chamber and an odor novel to the female (FFH, Furfuryl heptanoate) in the opposite chamber. Previous studies on *N. vitripennis* had examined the response of females in a four chamber olfactometer with the odor females were trained on in one chamber versus the opposite chamber left empty (Schurmann *et al.* 2009; Schurmann *et al.* 2012). Additionally, these studies had used previously frozen and thus dead hosts as a reward. Reward value can influence memory formation in parasitic wasps (Kruidhof *et al.* 2012) and dead hosts represent a poor quality host for *N. vitripennis* (Koppik, Thiel & Hoffmeister 2014). To complement our results and make them comparable to earlier investigations, we thus tested if drilling into a dead host also elicits long term memory formation in the HVRx strain. At the same time we investigated if the testing protocol (opposite chamber left empty or offered with a novel odor) influences memory retention.

Materials and methods

Control (odor but no host reward) and conditioning (1 × Drilling into a live or dead *Lucilia sericata* pupa) treatments lasted a maximum of 30 minutes, females that did not show the target behavior after 30 minutes were excluded from the experiment. Females were tested 1, 3 and 7 days after conditioning in a four chamber olfactometer as in the previous experiments if not stated otherwise. 50 mg (± 5 mg) of cinnamon was placed in one chamber and in the opposite chamber either a glass capillary (20 µl) half filled with FFH (Cinnamon – FFH) was placed or the chamber was left empty (Cinnamon – empty). For each time – treatment combination 6–15 replicates were made.

Results

The time span between treatment and testing did not influence the proportional walking time of females on the cinnamon odor field (GLM, binomial error distribution corrected for overdispersion, N = 130; time * treatment: $F_{6,118} = 0.669$, P = 0.6746, time: $F_{2,124} = 0.7484$, P = 0.475). But treatment had a significant influence on the proportional walking time (GLM, binomial error distribution corrected for overdispersion, N = 130, treatment: $F_{3,126} = 14.156, P < 0.001$). Conditioning (with live and dead hosts and for both testing treatments) led to a significantly higher proportion of time spent over the cinnamon field compared to control females (Odor control) (Figure 4.7, Table 4.5); females with a drilling experience on a dead hosts seem to have formed an LTM (up to at least 7 days) for cinnamon. Proportional walking time on cinnamon was not influenced by the experimental protocol (opposite chamber left empty or offered with a novel odor) (Figure 4.7, Table 4.5).

Table 4.5 Multiple comparisons of mean proportional walking times between the three treatments, P values are adjusted with the Tukey's test for multiple comparisons

Comparison	**z**	**P**
Odor control – Dead hosts (Cinnamon – FFH)	-5.560	< 0.001
Odor control – Live hosts (Cinnamon – FFH)	-4.732	< 0.001
Odor control – Live hosts (Cinnamon – empty)	-5.493	< 0.001
Dead hosts (Cinnamon – FFH) – Live hosts (Cinnamon – FFH)	0.546	0.948
Live hosts (Cinnamon – empty) – Live hosts (Cinnamon – FFH)	0.975	0.763
Dead hosts (Cinnamon – FFH) – Live hosts (Cinnamon – empty)	-0.509	0.957

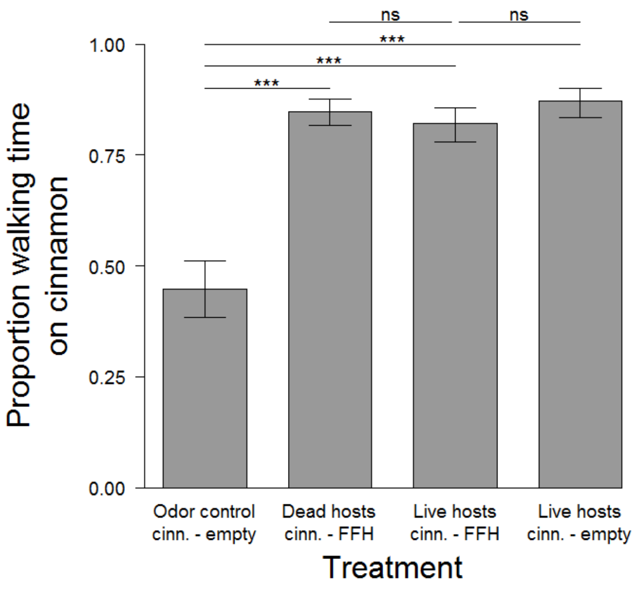

Figure 4.7 Estimates (± SE) of proportional walking time above the cinnamon field for control treatment ('Odor control') and for conditioning on either live or dead hosts ('Live hosts' or 'Dead hosts') and different testing treatments ('Cinnamon – empty' or 'Cinnamon – FFH'). HVRx females were conditioned on cinnamon on live/dead hosts and tested on cinnamon versus FFH/empty 1, 3 and 7 days after treatment. Time interval between treatment and testing had no significant influence on the proportion of time females spent walking over cinnamon and is not plotted here;
Abbreviations: cinn.: Cinnamon, FFH: Furfuryl heptanoate, ns: $P > 0.05$, *** $P < 0.001$ (P values of multiple comparisons were adjusted with Tukey's test)

5 Clutch size variation in the parasitic wasp *Nasonia vitripennis* in response to physiological state and the information content in host experience

Mareike Koppik, Andra Thiel & Thomas S. Hoffmeister

In preparation for submission to *PLoS ONE*

5.1 Abstract

In patchy environments, estimating overall habitat quality correctly is essential for obtaining adaptive decisions within patches. In insect parasitoids, females foraging for hosts within patches have repeatedly been shown to employ behavioral strategies that are in line with the use of information gained about patch density and patch quality. Yet, whether those behavioral strategies are based on information gained through the foraging process or whether they are merely a by-product of changes in the physiological state of an individual (e.g. due to egg laying) has rarely been tested. Using the gregarious parasitoid *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae), we tested the effect of the quality of hosts previously encountered on current clutch size decisions. Females were given the opportunity to examine and receive information about hosts under conditions ('host examination modes') that either allow for a change in information status only or in physiological state as well. Quality differences in previously encountered hosts did not affect clutch size decisions and thus a proxy of current habitat quality estimates within both host examination modes. In contrast, we found that the mode of host examination allowing for more information about hosts led to higher expectations of habitat quality in females. Further analyses showed that the higher estimate of habitat quality in females being exposed to a host for 2 hours (i.e. possible change in physiological state) was not due to egg laying. These findings are in line with studies on learning of host associated odors in *N. vitripennis,* where a prolonged host encounter but not egg laying per se leads to higher memory retention. We conclude that prolonged host encounters might provide females with additional information supporting information based decision making. Furthermore, we suggest that initial information processing in habitat quality estimation and in associative learning may use the same pathways in this parasitoid.

5.2 Introduction

In order to behave optimally in variable environments, foraging individuals have to keep track of the overall quality of the habitat (Ydenberg, Brown & Stephens 2007). Since a forager is not omniscient regarding the profitability of its environment it may use information about the distribution and profitability of resource patches encountered in the past to estimate the overall quality of its habitat. Theoretical models show that learning and retention of habitat parameters can improve foraging decision making over time (McNamara & Houston 1985; McNamara & Houston 1987). While many studies have been conducted on perceivable changes in habitat quality that trigger changes in the estimation of habitat quality in female parasitoids, these studies have mostly concentrated on patch time allocation (see Wajnberg 2006 for a review) with only few studies testing the effect on clutch size decisions (but see Rosenheim & Rosen 1991; Bezemer & Mills 2003). Furthermore, the influence of information and physiological state (e.g. egg load, age) is often difficult to disentangle (but see Rosenheim & Rosen 1991).

The quantitative framework of optimal patch exploitation given by Charnov states that an animal should leave the current patch when its “marginal capture rate in the patch [….] drops to the average capture rate for the habitat” (Charnov 1976, p 132). Thus optimal foraging decisions on patches require information about the profitability of the current patch as well as the overall habitat. Clutch size decisions in insects such as parasitoids can be treated as an optimal foraging problem (Iwasa, Suzuki & Matsuda 1984; Skinner 1985; Mangel 1987). Optimal clutch size per oviposition bout, with one of the most well-known studies done by Lack (Lack 1947) in birds, has also been studied in a variety of insect species considering the fecundity of resulting offspring instead of their mere survival (Charnov & Skinner 1984; Godfray, Partridge & Harvey 1991; Mangel, Rosenheim & Adler 1994). Here, optimal clutch size is the number of eggs that maximizes the fitness gain of the ovipositing female per oviposition bout. Theoretical models maximizing lifetime fitness gain are based on models of optimal clutch size and have been expanded to match the purposes of insects foraging for oviposition sites, by including e.g. egg load and mortality into the models (Iwasa, Suzuki & Matsuda 1984; Mangel 1987; Mangel 1989; Mangel & Heimpel 1998) using rate-maximization as well as dynamic state-variable approaches. One prediction of such models is that in parasitoids, maximizing lifetime fitness gain should lead to decreasing exploitation (smaller clutch sizes) of single hosts with increasing overall habitat quality.

There is a close link between foraging success and lifetime fitness gain in parasitoid females searching for hosts (Godfray 1994). This makes them attractive study systems since foraging should be a well-adapted trait and fitness consequences of individual decisions can be easily estimated. Female parasitoids adjust their foraging behavior in response to previous experiences as well as to their own state. Patch time allocation changes in response to the experienced encounter rates with or travel effort between patches, two proxies for patch distance, as well as in response to the experienced quality of patches (e.g. Boivin, Fauvergue & Wajnberg 2004; Thiel & Hoffmeister 2004; Tentelier, Desouhant & Fauvergue 2006; Thiel, Driessen & Hoffmeister 2006; Muratori, Boivin & Hance 2008; Louapre *et al.* 2011). As predicted by the Marginal Value Theorem (Charnov 1976), patch time on a given patch generally decreases with increasing habitat quality. Different habitat qualities also affect clutch size decisions (Rosenheim & Rosen 1991; Bezemer & Mills 2003). Additionally, information on possible competition for hosts can influence a female’s clutch size decision (Hoffmeister *et al.* 2000). Cox-regression models have proven to be a powerful tool to examine the influence of single events on the time allocation of parasitoid females (Wajnberg *et al.* 2003). These models have revealed the species specific influence of e.g. ovipositions and host rejections on patch time allocation (Wajnberg *et al.* 2003; Wajnberg 2006). Besides the effects of environment, foraging decisions are also influenced by a forager’s state like age (Elzinga, Harvey & Biere 2005), life expectancy (Roitberg *et al.* 1993) and egg load (Odendaal & Rausher 1990; Heimpel, Rosenheim & Mangel 1996; van Randen & Roitberg 1996; Migani, Ekesi & Hoffmeister 2014). Among these, egg load has repeatedly been shown to be a major factor driving foraging decisions in many insect species (see Minkenberg, Tatar & Rosenheim 1992 for a review).

Manipulations of previous experience often go along with changes in the physiological state of an individual impeding studies on the influence of information on decision making processes in insects (Rosenheim & Rosen 1991), thus only a few studies disentangled these two factors. An experiment on clutch size decisions in *Aphytis lingnanensis* Compere (Hymenoptera: Aphelinidae) revealed state and experience (information) based decisions, by manipulating egg load through size and rearing temperature of experimental females (Rosenheim & Rosen 1991). Studies on the effect of previous patch quality on habitat quality estimates in the parasitic wasp *Asobara tabida* (Hymenoptera: Braconidae) excluded habituation or egg load as forces driving subsequent foraging decisions (Thiel & Hoffmeister 2006). An investigation on travel time measurements in *A. tabida* concludes that a Bayesian-like updating mechanism is most likely to be present in this wasp again excluding factors like aging or egg load (Thiel 2011). Behavioral experiments on *Venturia canescens* (Hymenoptera: Ichneumonidae), on the other hand, showed that these wasps, depending on their natural habitat, use either the energy expenditure during flight or the waiting time between patch visits as a cue for patch distance (Liu, Bernstein & Thiel 2009), suggesting at least some influence of physiological state on decision making. Habitat quality estimation in this species seems to be based on the number of eggs laid on previous patches not the kairomone level therein (Froissart *et al.* 2012), again suggesting that not information on abundance of hosts but the physiological changes during egg laying might influence habitat quality estimates. A recent study on *Asobara tabida* indicates that learning and the use of short term memory is involved in habitat quality estimation in this wasp (Louapre & Pierre 2012), clearly suggesting an information based mechanism.

To elucidate the information use during foraging in the gregarious parasitoid *Nasonia vitripennis* (Hymenoptera: Pteromalidae) we investigated 1. whether the quality of a previously encountered host changes the habitat quality estimation of a female and 2. whether habitat quality estimation differs for different host examination modes (changing information state only or physiological state as well). We used clutch size decisions as a proxy for habitat quality estimation. *N. vitripennis* is a gregarious parasitoid, 1.0–3.5 mm in size, attacking pupae of a wide range of cyclorraphous Diptera (Whiting 1967). Females are concurrent host feeders (Rivero & West 2005) and their egg maturation and resorption is strongly dependent on host feeding (Edwards 1954a; Richard & Casas 2012). Investigations on the foraging behavior in this species have shown that females adapt their current clutch size and differ in their restlessness in response to low quality hosts (King & Rafai 1970; Werren 1984; Shuker *et al.* 2005; King & Ellison 2006; Koppik, Thiel & Hoffmeister 2014). Since it is still unclear what is perceived as a patch in

N. vitripennis (Shuker *et al.* 2007), the present study uses patches containing only a single host. We allowed females to either drill into a host ('host examination mode' allowing for changes in informational status only) or spend a two-hour period with a host ('host examination mode' allowing for changes in physiological state as well) of low, medium or high quality and measured their clutch size decision on the subsequent host as a proxy for their habitat quality estimate.

5.3 Materials and Methods

Study organisms

For the experiments we used the laboratory strain HVRx of *Nasonia vitripennis*. This is a strain from five different lines collected in 2001 in the Hoge Veluwe area in the Netherlands and cultured to maintain high genetic variability (van de Zande *et al.* 2014).Wasps were kept in an incubator at 25 °C and 60 % r.h. under a L16:D8 regime and reared on fly pupae of *Calliphora vomitoria* L. (Diptera: Calliphoridae). Hosts were obtained as maggots from a local pet shop and kept at 25 °C in saw dust until two days after pupation. Afterwards they were transferred to 4 °C until use.

Female wasps were sorted as pupae and kept individually in gelatine capsules (0.37 cm^3) until hatching. Freshly emerged females were kept singly in polystyrene vials (27 × 60 mm) and fed 10 % (vol/wt) sugar solution. On the first day every female was kept with a male to allow mating. On the second to fourth day females had access to one fresh standardized host (67.5–72.5 mg) each day, on the fourth day, however, the host was removed after 4 hours and females were deprived of hosts (for 18–20 hours) until the start of the experiment on the next day. This procedure allows females to mature and accumulate eggs. Female wasps were used for experiments five days after hatching.

For the experiments hosts were weighed on the day of pupation. We created three different host qualities for the pre-experience of the females. Good quality hosts were obtained by using large hosts (82.5–87.5 mg) kept at 25 °C until two days after pupation and stored at 4 °C until use. Medium quality hosts were obtained by using hosts (67.5–72.5 mg) kept at 25 °C until one day after pupation and parasitized by a female of a red-eyed mutant strain for 4 hours one day before the experiment. Low quality hosts were obtained the same way only that hosts were preparasitized four days before the experiment. Previous studies have shown that females add fewer or no eggs to previously parasitized hosts, which therefore represent medium/low quality hosts (King & Rafai 1970; Werren 1984; Shuker *et al.* 2005; Koppik, Thiel & Hoffmeister 2014).

Experiment

Experiments were conducted in a climatic chamber at a constant temperature of 25 ± 1 °C. Experiments consisted of a pre-experience phase and an experimental phase (Figure 5.1). In both phases females received a host in a closed petri dish (Ø 5 cm). The two phases were separated by a two-hour resting period, during which females were kept singly in polystyrene vials (27 × 60 mm) and fed 10 % (vol/wt) sugar solution.

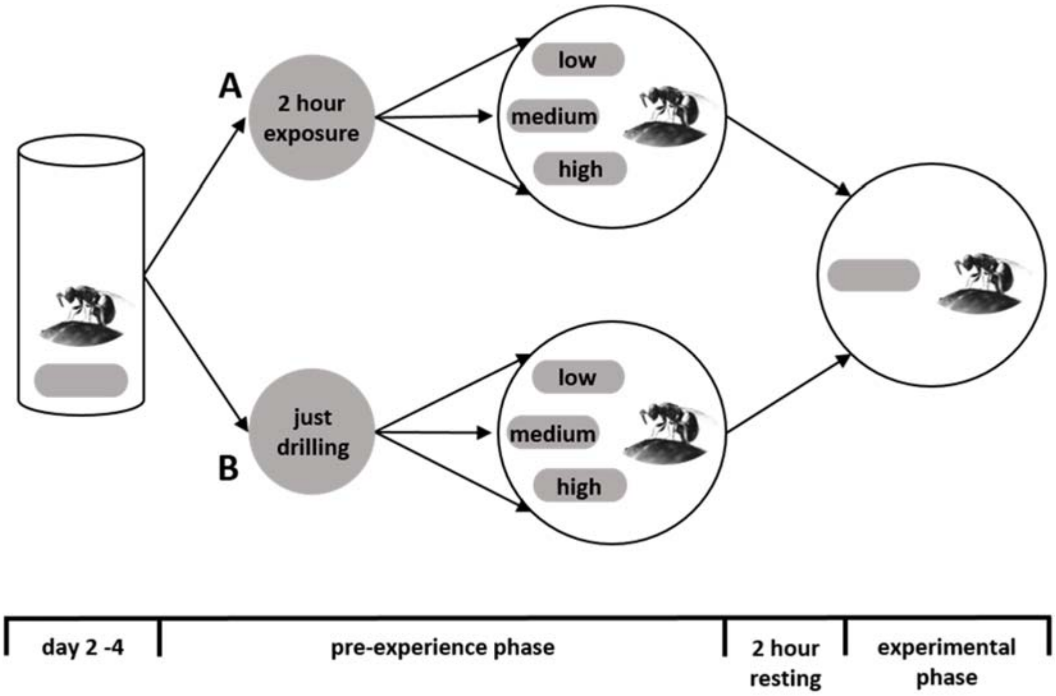

Figure 5.1 Experimental setup. Experimental females differed in their experience during the pre-experience phase. One half of the females was exposed to a host (*Calliphora vomitoria*) of low medium or high quality for 2 hours (**A**). The other half of the females was only allowed to drill into a host (*C. vomitoria*), again, of low, medium or high quality (**B**). After a 2 hour resting period clutch size decisions of all females were recorded on the same kind of host (*C. vomitoria* pupa weighing 52.5–57.5 mg).

During the pre-experience phase there were two different ‘host examination mode’ groups: 1. only drilling. Here females were only allowed to drill into a host and were afterwards gently brushed off the host, therefore females could sense the quality of the host but could not host feed or lay eggs; 2. two-hour exposure. Here females were allowed to spend two hours with the host, including egg laying and host feeding. Within each ‘host examination mode’ group females were divided into three host quality groups either receiving a high, medium or low quality host during the pre-experience phase.

During the experimental phase females from all six groups were treated the same. Each female received one host (52.5–57.5 mg) and she was constantly observed until she decided to abandon the host (5 minutes off the host) or until 10 pm (end of light phase in the rearing incubator). Afterwards females were transferred to ice (to stop egg maturation) and at the end of the day frozen to determine the number of remaining mature eggs inside their ovaries. Egg load was defined as the number of eggs that were available to the female during the experimental phase: remaining mature eggs plus eggs laid on the second host. In the analyses ln-transformed egg loads were used.

All hosts of the pre-experience and experimental phase were kept at 25 °C for 15 days to count hatching offspring. Afterwards all hosts were carefully opened to also check for dead, diapausing or not fully developed offspring. Trials with hosts that turned out to be unsuitable for parasitization were excluded from the analysis. For each ‘host examination mode’ – ‘host quality’ combination 18–21 replicates entered the analysis.

Statistical analysis

Statistics were performed with the software R 3.0.2 (R Core Team 2013) and package *car* (Fox & Weisberg 2011) using Generalized Linear Models (GLM, Fox & Weisberg 2011). The respective error distributions are given with the test results.

Graphs were made using the software R 3.0.2 (R Core Team 2013) and packages *gplots* (Warnes 2012), *sfsmics* (Maechler & al. 2012) and *calibrate* (Graffelman 2012).

5.4 Results

Influence of current host quality on clutch size

By allowing females to produce egg clutches during the two-hour exposure on hosts in the pre-experience phase we can test if females were able to sense the quality of the offered host and would respond with clutch size variation. We found clutch size to be significantly influenced by the quality of the current host (GLM with Poisson error distribution corrected for overdispersion, N = 60, $F_{2, 57} = 29.618$, $P < 0.001$). Females laid significantly larger clutches on hosts of higher quality compared to medium or low quality hosts (Figure 5.2).

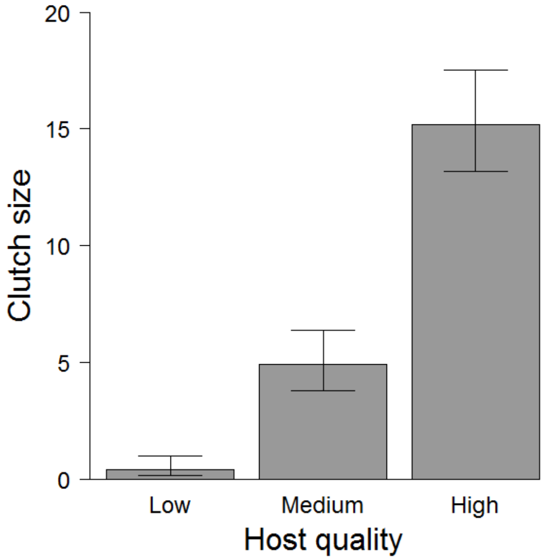

Figure 5.2 Influence of current host quality on clutch size decisions. During a 2 h time period females laid significantly more eggs on high quality hosts than on lower quality hosts (N = 60, P < 0.001), estimates (± SE) derived from the statistical model

Influence of previous experience on clutch size

The influence of previous experiences on clutch size was analyzed using clutch size data of the experimental phase. Here females with different experiences (only drilling into/two-hour exposure to either a low, medium or high quality host) had access to the same kind of host until they abandoned that host. Since females with different experiences should have different egg loads, egg load was always included in the models to control for effects derived from a female's egg state. Since some trials (11 out of 118) were terminated by the experimenter at 10 pm, an additional variable (censored) was introduced to check for any influence of artificial termination of the experiment.

Table 5.1 Results of the statistical analysis of clutch size decisions on the second host (GLM, Poisson error distribution, N = 118).

Variable	**d.f.**	**χ^2**	**P**
ln(egg load) * host examination mode * previous host quality	2	0.465	0.793
host examination mode * previous host quality	2	2.119	0.347
ln(egg load) * previous host quality	2	4.778	0.092
ln(egg load) * host examination mode	1	10.302	0.001
previous host quality	2	0.630	0.730
host examination mode	1	2.704	0.100
ln(egg load)	1	108.319	< 0.001
censored	1	1.357	0.244

Deviance based R_D^2 = 0.41 of the minimal adequate model: clutch size ~ ln(egg load) * host examination mode

Clutch size was influenced by the 'host examination mode' on the previous host but was not significantly influenced by the quality of the previous host; termination of the experiment (censored) had no significant influence on clutch size (Table 5.1). There was a strong positive relationship between egg load of the females and the clutch size they laid (Figure 5.3). Egg load even influenced the way the 'host examination mode' affected clutch size, as can be seen by the significant interaction between these two terms (Table 5.1). At low egg loads females laid smaller clutches after a drilling experience on the previous host than after a two-hour exposure, but the pattern was reversed at high egg loads (Figure 5.3).

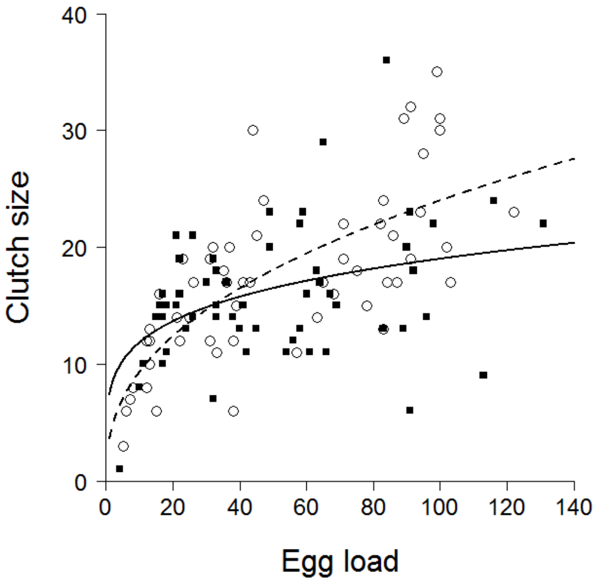

Figure 5.3 Influence of previous host examination mode on current clutch size decisions. Clutch size decisions of females as a function of their egg load. Females that spent 2 hours with the previous host laid bigger clutches at low egg loads and smaller clutches at high egg loads compared to females that only drilled into the previous host, raw data and regression lines derived from the statistical model (2 hour exposure: filled squares, solid line; only drilling: open circles, dashed line).

To separate the effect of experience with hosts and changes in physiological state due to egg laying, we divided the dataset into females that had laid eggs on the first host (only females of the 2 hour exposure group) and females that had not laid eggs on the first host (all females of the drilling group and part of the 2 hour exposure group). Within the group of females that had laid no eggs previously there was still a significant influence of 'host examination mode' on clutch size (Table 5.2), the overall pattern (Figure 5.4A) remained the same compared to the results of the full data set (Figure 5.3).

Table 5.2 Results of the statistical analysis of clutch size decisions on the second host, when females did not lay eggs on the first host (GLM, Poisson error distribution, N = 88).

Variable	**d.f.**	**χ^2**	**P**
ln(egg load) * host examination mode	1	4.841	0.028
host examination mode	1	0.135	0.713
ln(egg load)	1	107.571	< 0.001
censored	1	0.464	0.496

Deviance based R_D^2 = 0.47 of the minimal adequate model: clutch size ~ ln(egg load) * host examination mode

Within the group of females that had laid eggs on the first host we tested for the effect of number of eggs laid on first host (which might be used as a measurement of host quality (Froissart *et al.* 2012)) on clutch size on the second host. There was no significant effect of number of eggs laid previously, clutch size only depended on egg load of the female (Table 5.3), where clutch size increased with increasing egg loads (Figure 5.4B).

Table 5.3 Results of the statistical analysis of clutch size decisions on the second host, when females laid eggs on the first host (GLM, Poisson error distribution, N = 30).

Variable	**d.f.**	**χ^2**	**P**
ln(egg load) * no. of eggs laid previously	1	0.061	0.804
no. of eggs laid previously	1	0.548	0.459
ln(egg load)	1	6.776	0.009
censored	1	0.514	0.474

Deviance based R_D^2 = 0.13 of the minimal adequate model: clutch size ~ ln(egg load)

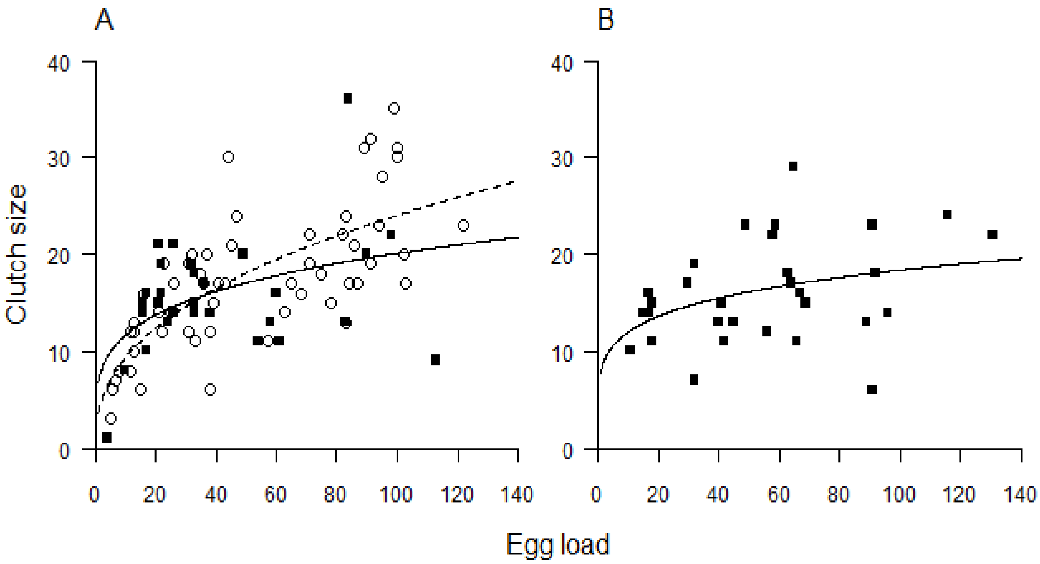

Figure 5.4 Previous experience intensity not egg laying influences subsequent clutch size decision. Dividing the full dataset into females that laid no eggs on the first host (**A**; N = 88) and females that laid eggs (**B**; N = 30) reveals that egg laying is not the cue that triggers changes in habitat quality estimation. Even though females laid no eggs on the first host during the two-hour exposure pre-experience they still reduced their clutch size compared to females of the only drilling pre-experience (**A**) (raw data and regression lines derived from the statistical model; only drilling: open circles, dashed line; two-hour exposure: filled squares, solid line). Furthermore, within the group of females that laid eggs on the first host, clutch size did not depend on number of eggs laid previously (P = 0.459, Table 5.3) but only increased with increasing egg load of the female, raw data and regression line derived from the statistical model (**B**)

5.5 Discussion

Parasitoid females obviously use prior experiences to update their habitat quality estimate and adjust their foraging behavior (e.g. Rosenheim & Rosen 1991; Bezemer & Mills 2003; Thiel & Hoffmeister 2004; Liu, Bernstein & Thiel 2009). Still, the extent to which information and physiological state contribute to the decision making process has not been fully understood yet. Using clutch size decisions in the gregarious parasitoid *Nasonia vitripennis* as a proxy for habitat quality estimation, we investigated 1. whether the quality of a previously encountered host changes the habitat quality estimation of a female and 2. whether habitat quality estimation differs for different modes of host examination.

As expected clutch size varied with the quality of the current host in an adaptive manner (Figure 5.2). Lower quality hosts produced fewer offspring and this reduction in offspring number usually is a result of the female's clutch size decision (Koppik, Thiel & Hoffmeister 2014). This shows that females were able to sense the quality of the host while parasitizing it. However, females did not significantly alter their clutch size decisions on the subsequent host in response to previous host quality (Table 5.1). This might indicate that changes in host/patch quality is not used in *N. vitripennis* to change the estimate of habitat quality, which is in contrast to many other species studied (e.g. Rosenheim & Rosen 1991; Bezemer & Mills 2003; Wajnberg 2006; Louapre *et al.* 2011). However, a similar phenomenon has been shown for the parasitic wasp *Lysiphlebus testaceipes*, where the first patch encounter seems to set the habitat quality estimate of a female rather than a constant updating of information during foraging (Tentelier, Lacroix & Fauvergue 2009). Low quality hosts in our study have been obtained by pre-parasitization of hosts, a feature that changes subsequent foraging behavior in several species (Roitberg *et al.* 1992; Michaud & Mackauer 1995). However, it does not change habitat quality estimation in the parasitoid *Asobara tabida* (Thiel & Hoffmeister 2006). *A. tabida* is not able to distinguish self- and conspecifically parasitized larvae, making parasitization status of the host an unimportant information in this species (Thiel & Hoffmeister 2006). *N. vitripennis* is able to distinguish between the two kinds of parasitized hosts, but only under certain circumstances (King 1992). Thus, it remains unclear if parasitization status of a previous hosts should be used as a cue for habitat quality assessment in this species. The interaction ‘ln(egg load) * previous host quality’, though, has been close to the 5 % significance level within our study (P = 0.092, Table 5.1), such that a small biological effect of previous host quality cannot be excluded with certainty. Nevertheless, we conclude that previous host quality at least had no major effect on habitat quality estimation in *N. vitripennis*.

In contrast to *Venturia canescens*, which adjusts habitat quality estimation based on the number of eggs laid on previous patches (Froissart *et al.* 2012), we did not find such a mechanism in *N. vitripennis*. A female’s clutch size decision was not influenced by the number of eggs laid on the previous host (Table 5.3). As a generalist *N. vitripennis* parasitizes a broad range of dipteran hosts that may be found on carcasses or birds’ nests (Abraham 1985; Voss, Spafford & Dadour 2009). These communities of flies may be mixed (Daoust *et al.* 2012) such that quality of hosts may vary greatly even within one host patch (Peters 2010). Accordingly, the quality of one host encountered might not have a high predictive value for the quality of other hosts around and might therefore not be used by females to estimate habitat quality.

In contrast to previous host quality, the mode of host examination on the previous host had a significant influence on the subsequent clutch size decision (Table 5.1). At high egg loads females that had only drilled into the host but were not allowed to parasitize or host feed laid more eggs onto the next host than females that were exposed to that host for 2 hours (Figure 5.3). Since we corrected for egg load in our analysis these changes are most likely derived from the different host examination modes on the previous host. Consequently, different modes of host examination seem to have led to different estimates of habitat quality even though the actual quality of the habitat was the same. Both groups had the same number and frequency of host encounters, which should lead to the same habitat quality estimate, but females seem to have valued the two types of experiences differently. This is in line with findings in studies on associative learning in *N. vitripennis* (Schurmann *et al.* 2009; Schurmann *et al.* 2012). Depending on the strain, females form longer lasting memories or have a higher memory retention after being allowed to host feed/lay eggs compared to females that were only allowed to drill into the host during associative learning of an odor (Schurmann *et al.* 2009; Schurmann *et al.* 2012; chapter 4). Even though egg laying induces various changes in the transcriptome of

N. vitripennis females (Pannebakker *et al.* 2013), the better habitat quality estimate in the two-hour exposure group was not due to egg laying. Excluding females that had laid eggs on the first host did not change the qualitative outcome of the analysis (Table 5.2, Figure 5.4A). Therefore, information use rather than a change in physiological state, seems to have triggered the changes in the habitat quality estimate of females. The same pattern can be found in associative learning in *N. vitripennis* (Hoedjes & Smid 2014). Longer lasting experiences on the host might provide the female with additional information about the suitability of the host and might therefore represent a more reliable cue about a host encounter. This might explain why females value longer lasting host encounters higher, both in habitat quality estimation and learning of host associated odors. Our experiments suggest that *N. vitripennis* females might use the same pathways in initial information processing during associative learning and during updating of their habitat quality estimate. This is in line with the study on *Asobara tabida* that shows that short term memory might be involved in habitat quality estimation in this species (Louapre & Pierre 2012).

Besides previous experience, egg load played a major role in the clutch size decisions of females with higher egg loads leading to higher clutch sizes. This is in line with theoretical models of clutch size decisions stating that females should lower the number of eggs laid per host at low egg loads to avoid becoming egg limited (Iwasa, Suzuki & Matsuda 1984; Mangel 1987). But egg load did also affect the effect of host examination mode (significant interaction between egg load and ‘host examination mode’, Table 5.1 and 5.2). While at high egg loads females with the drilling experience produced bigger clutches than those in the 2 hour exposure group, this pattern was reversed at low egg loads (Figure 5.3 and 5.4A). We hypothesize that this phenomenon is related to the fact that females in the two-hour exposure group also had the opportunity to host feed during the pre-experience phase. Egg maturation in *N. vitripennis* is strongly dependent on host feeding (Edwards 1954a; Richard & Casas 2012). Most likely egg maturation rate was higher at the start of the experimental phase in those females that were able to host feed during the pre-experience phase. Therefore these females might have laid a slightly higher fraction of their available eggs at low egg loads since they already could start to mature further eggs and expected to have more eggs available sooner than females, which lacked the previous host feeding opportunity.

To conclude, we showed that *N. vitripennis* females did not use the number of eggs laid on a previous host to make clutch size decisions and that previous host quality did at least not have a major impact on current clutch size decisions. But females used their current egg load and probably egg maturation rate to adjust the number of eggs laid on the host, clearly showing an influence of physiological state on clutch size decisions. Most importantly, different modes of host examination on a previous host without egg laying led to different habitat quality estimates, suggesting an information based component in foraging decisions of *N. vitripennis* females. Associative learning in this species shows similar features. Here, different types of host examination lead to different memories or levels of memory retention (Schurmann *et al.* 2009; Schurmann *et al.* 2012; chapter 4). These parallels might indicate that initial information processing in habitat quality estimation uses the same pathways as in associative learning.

5.6 Acknowledgements

This study was funded by the Central Research Development Fund, University of Bremen, grant 02/125/10 to AT.

6 Wait and see: time allocation under egg limitation in the synovigenic parasitoid *Nasonia vitripennis*

Mareike Koppik, Thomas S. Hoffmeister & Andra Thiel

In preparation for submission to *Behavioral Ecology*

6.1 Abstract

Decisions on the allocation of essential resources, such as time and eggs, are crucial for animals and are impacting their lifetime fitness gain. In many insect species the decisions on where and how many eggs to lay is known to be influenced by the current supply of mature eggs. Models of optimal behavior integrating the egg supply of a female usually deal with the optimal allocation of those eggs, e.g. optimal clutch size. However, time might still be a limited resource and decisions about time allocation have to be made by individuals as well. The question how time allocation decisions are made by female insects under egg limitation has seldom been addressed. Here we show that females of the parasitoid *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) can transiently become egg limited. With decreasing egg loads females increased the time spent on a host, most likely to mature and lay further eggs. This demonstrates that females adapted their time allocation decisions to their current egg state. This in turn may lead to the production of more eggs to be laid into the current host, thus the final clutch size may exceed the number of eggs that have been available to the female when first encountering the host.

6.2 Introduction

Parasitoid females searching for hosts can be limited in their lifetime reproductive success by the number of suitable hosts available, by the amount of time to search for and parasitize hosts and/or by the number of mature eggs to lay into/onto the encountered hosts (Godfray 1994). These limitations led to the development of theoretical models of optimal foraging behavior in parasitoids that maximize fitness gain per host, unit of time or egg, respectively (Charnov 1976; Charnov & Skinner 1984; Iwasa, Suzuki & Matsuda 1984; Mangel, Rosenheim & Adler 1994). A model by Charnov and Skinner (1988) shows that under certain circumstances it can be optimal for a parasitoid female to remain on a host and mature further eggs after parasitization. Though it has been reported for parasitoid females to remain on the host after parasitization especially as their egg load is low (Donaldson & Walter 1988), the relationship between habitat quality, egg load and waiting time on a host has rarely been tested to date.

Remaining on a host to mature eggs should only occur in parasitoids that mature eggs throughout their life (synovigenic species) and that may become egg limited. Due to the continuous egg maturation synovigenic species might not be limited by their lifetime egg supply, but they may be temporarily egg limited (transient egg limitation) depending on their host encounter and egg maturation rate (Charnov & Skinner 1988; Casas *et al.* 2000; Rosenheim, Heimpel & Mangel 2000; Casas *et al.* 2009). High host encounter rates may lead to egg limitation because the oviposition opportunities exceed the egg supply of a female. Egg resorption may additionally lead to egg limitation at very low host encounter rates (Rosenheim, Heimpel & Mangel 2000). Whether egg limitation should evolve at all or if all species should evolve an egg maturation strategy that makes them time limited rather than egg limited is an ongoing debate (Rosenheim 1996; Sevenster, Ellers & Driessen 1998; Ellers, Sevenster & Driessen 2000; Rosenheim 2011). However, egg load has been recognized as an important factor driving foraging decisions in many insects in empirical as well as theoretical studies (Iwasa, Suzuki & Matsuda 1984; Mangel 1989; Minkenberg, Tatar & Rosenheim 1992). Several field studies have demonstrated that parasitoid females can become egg limited under natural conditions (Heimpel, Mangel & Rosenheim 1998; Heimpel & Rosenheim 1998; Casas *et al.* 2000; Rosenheim *et al.* 2008; Segoli & Rosenheim 2013).

Models on optimal clutch size decisions in gregarious parasitoids, which integrate the egg supply of a female as a limiting factor show that females should reduce the number of eggs laid depending on their current egg supply (Iwasa, Suzuki & Matsuda 1984; Mangel, Rosenheim & Adler 1994). The maximum clutch size in these models is usually the clutch size that maximizes the fitness gain per host for the ovipositing female (throughout this paper referred to as Lack Clutch Size) (Parker & Courtney 1984) and thus should only be laid if a female encounters just a single host in her lifetime or if there would be no trade-off between current and future reproductive success (Godfray, Partridge & Harvey 1991; Godfray 1994, p 100). The Lack Clutch Size theory in parasitoids has been adapted from the original theory developed for birds by Lack (1947). Several studies estimated the Lack Clutch Size of parasitoids in the laboratory (Charnov & Skinner 1984; Takagi 1985; Hardy, Griffiths & Godfray 1992; Vet *et al.* 1994; Zaviezo & Mills 2000). However, a study on clutch size decisions in the parasitoid *Mastrus ridibundus* (Hymenoptera: Ichneumonidae) demonstrated that the Lack Clutch Size estimates under laboratory conditions not necessarily match the estimates from field data (Bezemer & Mills 2003). Estimates under laboratory conditions overestimated the Lack Clutch Size, which may be explained by the fact that female fitness measures under laboratory conditions do not include size dependent host finding success (Bezemer & Mills 2003). Still, Lack Clutch Size estimations from the laboratory provide useful information to understand female clutch size decisions at least providing the upper limit for optimal clutch sizes.

For females that are limited by their current egg load theoretical models predict that it is suboptimal to lay the Lack Clutch Size (Godfray 1994). These female should rather reduce their clutch size to be able to deposit some of their eggs elsewhere in a new clutch, where they will provide a larger fitness return (Iwasa, Suzuki & Matsuda 1984; Mangel, Rosenheim & Adler 1994). Usually, the fitness return per egg is monotonically reduced with every additional egg laid – because the developing offspring competes for resources (Godfray 1994) – as long as no hump-shaped fitness relationship exists due to an Allee effect (Stephens, Sutherland & Freckleton 1999). Yet, the latter is seldom observed in parasitoids. Egg load dependent clutch size decisions should be selected to fall into the range between maximizing fitness gain per host (Lack Clutch Size) and maximizing fitness gain per egg (single egg clutches, given there are no Allee effects (Stephens, Sutherland &

Freckleton 1999)) (Godfray, Partridge & Harvey 1991). Models on oviposition decisions calculate the optimal clutch size of a female based on her current egg load, her environment and the fitness return for the given host (Iwasa, Suzuki & Matsuda 1984; Mangel, Rosenheim & Adler 1994). A model by Charnov and Skinner (1988), however, proposes that females with low egg loads in habitats with variable inter host travel times might not leave the host after oviposition to search for a new one, but instead stay on the host to mature and lay additional eggs. Some studies have reported that females engage in long resting periods near the hosts at low egg loads to mature further eggs (Donaldson & Walter 1988; Fernando & Walter 1999) but this phenomenon has rarely been investigated. The model by Charnov and Skinner (1988) predicts that females with low egg loads should stay on the host to mature and lay further eggs until a threshold time is reached. This threshold time depends on travel times between hosts and the average host value in the habitat of the female.

To investigate the relationship between habitat quality, egg load and waiting time on a host we used the parasitoid *Nasonia vitripennis*, a highly synovigenic gregarious parasitoid. Edwards (1954b) reported the anecdotal observation that *N. vitripennis* females remain on the host for long resting periods especially at low egg loads, which suggests egg limitation in this species. To support this assumption we first analyzed egg maturation rate and Lack Clutch Size on a common host (*Calliphora vomitoria* L. (Diptera: Calliphoridae)). We then analyzed the time allocation of females on a host under varying average host values in the environment. This allowed us to analyze the relationship between egg load and resting time on the host, and additionally test the prediction that this relationship is influenced by the average host value in the habitat.

6.3 Materials and methods

Study organisms

For the experiments we used the laboratory strain HVRx of *Nasonia vitripennis*. This is a strain from five different lines collected in 2001 in the Hoge Veluwe area in the Netherlands that was cultured to maintain a high genetic variability (van de Zande *et al.* 2014). *N. vitripennis* attacks a wide range of cyclorraphous fly pupae (Whiting 1967). Females are concurrent host feeders, 1.0–3.5 mm in size (Whiting 1967) with a lifespan of approximately 15 days (Davies 1975). Wasps were kept in an incubator at 25 °C and 60 % r.h. under a L16:D8 regime and reared on fly pupae of *Calliphora vomitoria*. Hosts were obtained as maggots from a local pet shop and kept at 25 °C in saw dust until two days after pupation. Afterwards they were transferred to 4 °C until use.

Egg maturation rate, longevity and Lack Clutch Size determination

To determine size dependent fitness components, females of different sizes were created by letting five females parasitize one host for different time spans (30 min to 24 hours). From the resulting female offspring 1 or 2 of the females that hatched from one host (variable: ‘Host of origin’) were used in the experiment. Those females were provided with honey and four fresh hosts every day until death. For each female, age at death, age at the end of the reproductive period, lifetime offspring production, average daily offspring production (lifetime offspring production/age at end of reproduction), and female size (estimated as length of the left hind tibia after death) was determined.

To determine clutch size dependent female offspring size, clutches in the range from 1 to 54 eggs produced on 2 day old *C. vomitoria* hosts weighing 52.5–57.5 mg (on the day of pupation) were used. Length of the left hind tibia of all female offspring was measured and the arithmetic mean of tibia length for female offspring from one host was used in the analysis.

Time allocation experiment

Female wasps were sorted as pupae and kept individually in gelatine capsules (0.37 cm^3) until hatching. Freshly emerged females were kept singly in polystyrene vials (27 × 60 mm) and fed 10 % (vol/wt) sugar solution. On the first day every female was kept with a male to allow mating. On the second to fourth day females had access to one fresh standard host (67.5–72.5 mg) each day. However, on the fourth day the host was removed after 4 hours and females were deprived of hosts (for 18–20 hours) until the start of the experiment on the next day. This procedure allows females to mature and accumulate eggs. Female wasps were used for experiments five days after hatching.

Hosts used for the experiment were weighed on the day of pupation. We created three different host qualities for the pre-experience of the females that manipulated average host quality in the habitat. 1) Good quality hosts were obtained by using large hosts (82.5–87.5 mg) kept at 25 °C until two days after pupation and stored at 4 °C until use. 2) Medium quality hosts were obtained by using hosts (67.5–72.5 mg) kept at 25 °C until one day after pupation and parasitized by a female of a red-eyed mutant strain for 4 hours one day before the experiment. 3) Low quality hosts were obtained the same way as medium quality hosts only that hosts were preparasitized four days before the experiment. Previous studies have shown that females add fewer or no eggs to previously parasitized hosts, which therefore represent medium/low quality hosts (King & Rafai 1970; Werren 1984; Shuker *et al.* 2005; Koppik, Thiel & Hoffmeister 2014).

Experiments were conducted in a climatic chamber at a constant temperature of 25 ± 1 °C. Experiments consisted of a pre-experience phase and an experimental phase (Figure 5.1, chapter 5). In both phases females received a host in a closed petri dish (Ø 5 cm). The two phases were separated by a 2 hour resting period for the females, during which they were kept singly in polystyrene vials (27 x 60 mm) and fed with 10 % (vol/wt) sugar solution.

During the pre-experience phase there were two different ‘host examination mode’ groups: 1. only drilling and 2. two-hour exposure (see Table 6.1). In the first host examination mode, females were only allowed to drill into a host (until full insertion of the ovipositor into the host puparium) and were afterwards gently brushed off the host, therefore females could sense the quality of the host but could not host feed or lay eggs.

In the second ‘host examination mode’, females were allowed to spend two hours with the host, including egg laying and host feeding. Within each of the ‘host examination modes’ females were divided into three groups either receiving a high, medium or low quality host during the pre-experience phase, leading to a crossed design of ‘host examination mode’ and ‘host quality’ experienced. All females were constantly observed during the pre-experience phase and their behavior (Table 6.1) was recorded using ‘The Observer XT’ (Noldus, Wageningen, NL).

During the experimental phase females from all six treatment groups were treated identically. Each female received one host (52.5–57.5 mg) and was constantly observed until she decided to abandon the host (5 minutes off the host) or until 10 pm (end of light phase in the rearing incubator). The behavior (Table 6.1) was recorded using ‘The Observer XT’ (Noldus, Wageningen, NL). Afterwards females were transferred to ice (to stop egg maturation) and at the end of the day frozen to determine the number of remaining mature eggs inside their ovaries later on. We estimated initial egg load (at the beginning of the experimental phase) from the mature eggs found inside their ovaries upon dissection plus eggs laid during the experimental phase minus matured eggs during the experimental phase (using the egg maturation function determined earlier).

Table 6.1: Behavior of females recorded during experiments (also see chapter 2.3)

Behavior	Description
Off	Females are off the host within the petri dish
Drumming	Females explore the host by walking on the host accompanied by alternating movements of the antennae
Tapping	Females explore the host’s surface with the tip of their abdomen
Drilling	Females drill a hole into the host puparium with their ovipositor
Oviposition	Females have their ovipositor fully inserted into the host either laying eggs or building a feeding tube
Host feeding	Females feed from the host’s body fluids through a feeding tube
Resting	Females remain on the host either resting (sitting still) or grooming

All hosts of the pre-experience and experimental phase were kept at 25 °C for 15 days to count hatching offspring. Afterwards all hosts were carefully opened to also check for dead, diapausing or not fully developed offspring. Trials with hosts that turned out to be unsuitable for parasitization were excluded from the analysis as well as trials that were terminated by the experimenter at 10 pm (N = 11). For each 'host examination mode' – 'host quality' combination 15–20 replicates entered the analysis.

Statistical analysis

Statistics were performed with the software R 3.1.0 (R Core Team 2014) and package *car* (Fox & Weisberg 2011) using Generalized Linear Models (GLM, Fox & Weisberg 2011) and package *lme4* (Bates *et al.* 2014) using Generalized Linear Mixed Models (GLMM). The respective error distributions are given with the test results.

Graphs were made using the software R 3.1.0 (R Core Team 2014) and packages *gplots* (Warnes *et al.* 2014) and *Cairo* (Urbanek & Horner 2013).

6.4 Results

Egg maturation rate and longevity

Average daily egg maturation rate was positively influenced by the size of the female (Figure 6.1). The size of a female measured by her hind tibia length had a significant effect on the number of eggs laid per day during her reproductive life span (GLMM with Gaussian error distribution and 'Host of origin' as a random term, N = 88, $\chi^2_1 = 102.2$, $P < 0.001$). Age at the end of reproduction and age at death were both significantly influenced by female size (GLMM with Poisson error distribution corrected for overdispersion and 'Host of origin' as a random term, N = 88, $\chi^2_1 = 10.599$, $P = 0.001$ and $\chi^2_1 = 11.945$, $P < 0.001$, respectively) with larger females living and reproducing for a longer time. Mean age at the end of the reproductive period was 17.01 days, while mean age at death was 20.29 days.

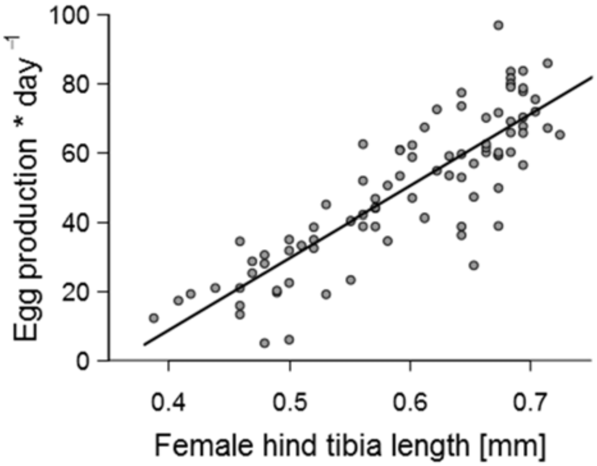

Figure 6.1 Influence of female size on daily egg maturation: *Nasonia vitripennis* females with a smaller hind tibia length laid fewer eggs per day during their reproductive life span than females with a larger hind tibia length when continuously provided with four fresh hosts every day; raw data and regression line derived from the statistical model

Lack Clutch Size

To estimate the fitness costs related to clutch size, the influence of clutch size on the size of the resulting female offspring was analyzed using the average hind tibia length (in mm) of all female offspring per clutch on hosts of *Calliphora vomitoria* weighing 52.5–57.5 mg. Larger clutches generated significantly smaller offspring (GLM with Gaussian error distribution, N = 162, $F_{1,160} = 192.08$, $P < 0.001$) with:

$$\textit{tibia length} = 0.7214625 - 0.0044750 * (\textit{clutch size}) \qquad \text{(Figure 6.2a).}$$

The influence of clutch size on the sex ratio (proportion males) of the clutch was analyzed using only single foundress clutches with at least one female offspring and no diapausing offspring. Clutch size had a significant negative influence on sex ratio (GLM with Binomial error distribution, N = 131, $\chi^2_1 = 5.601$, P = 0.018) with:

$\text{sex ratio} = 1 / (1 + 1 / \exp[-1.11536 - 0.02621 * (\text{clutch size})])$ (Figure 6.2b).

Assuming single foundress clutches, the number of grand offspring corresponds to the number of offspring that the females of a clutch produce, therefore the following proportions of females per clutch were used for the calculation of the Lack Clutch:

$\text{average proportion females} = 1 / [1 + 1 / \exp(1.60836)] = 0.8331836$

$\text{clutch size dependent proportion females} = 1 / (1 + 1 / \exp[1.115363 + 0.026215 * (\text{clutch size})])$

The influence of female size on her lifetime fecundity was analyzed using the hind tibia length (in mm) of the female and her total offspring production. Female size showed a significant positive relationship with her lifetime fecundity (GLMM with Poisson error distribution corrected for overdispersion and ‘Host of origin’ as a random term, N = 88, $\chi^2_1 = 41.962$, $P < 0.001$) with:

$\text{lifetime fecundity} = \exp[1.9314 + 7.7448 * (\text{tibia length})]$ (Figure 6.2c).

The Lack Clutch Size maximizes fitness gain per host, possible grand offspring production per host was used as a proxy for fitness. Either the average proportion of females (1) or the proportion of females as a function of clutch size (2) was used to estimate number of grand offspring per host (in the offspring generation) as a function of clutch size:

(1) $\text{no. grand offspring} = 1 / [1 + 1 / \exp(1.60836)] * (\text{clutch size}) * \exp(1.9314 + 7.7448 * [0.7214625 - 0.0044750 * (\text{clutch size})])$

(Figure 6.2d, dashed line),

(2) $\text{no. grand offspring} = 1 / (1 + 1 / \exp[-1.11536 - 0.02621 * (\text{clutch size})]) * (\text{clutch size}) * \exp(1.9314 + 7.7448 * [0.7214625 - 0.0044750 * (\text{clutch size})])$

(Figure 6.2d, solid line).

The clutch size that maximizes fitness gain per host can be calculated using the first derivative of the above functions with respect to clutch size. This results in a Lack Clutch Size of 28.85 eggs per host (*C. vomitoria*, 52.5–57.5 mg) using an average proportion of females (equation 1). A slightly higher Lack Clutch Size of 31.85 eggs per host (*C. vomitoria*, 52.5–57.5 mg) is estimated using clutch size dependent proportion of females (equation 2), since number of reproducing daughters increased with clutch size.

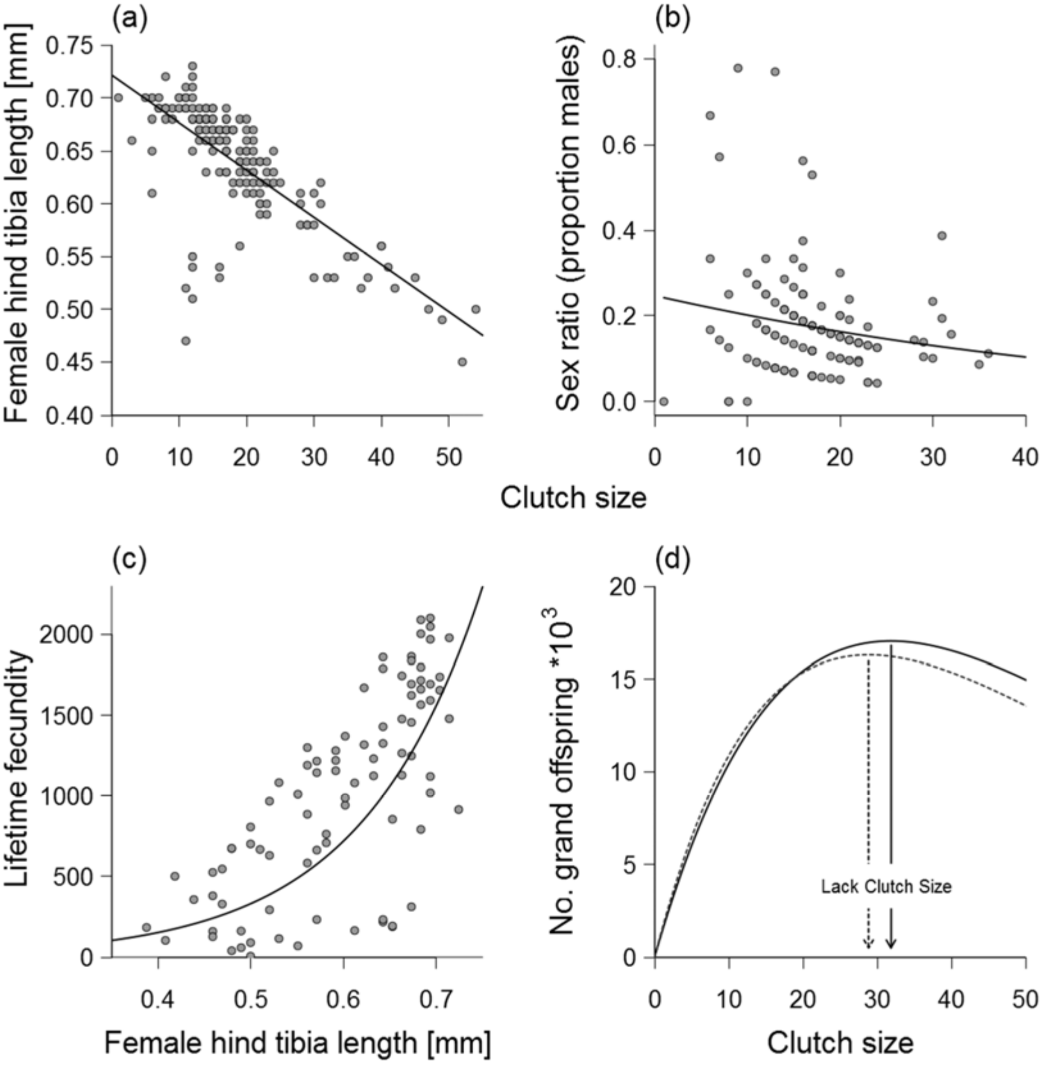

Figure 6.2 : *Nasonia vitripennis* Lack Clutch Size determination for *Calliphora vomitoria* hosts (52.5–57.5 mg), raw data and regression lines derived from the statistical models **a)** average female offspring size decreases with increasing clutch size **b)** sex ratio (as proportion of males) also decreases with increasing clutch size **c)** a decrease in female size results in a decrease in her lifetime fecundity **d)** using the above relationships results in a concave function for grand offspring production as a function of clutch size per host in the offspring generation (solid line), the curve slightly changes when an average proportion of females is used (dashed line) rather than a clutch size dependent proportion of females, which in turn results in a slightly smaller Lack Clutch Size

Time allocation

The influence of current host quality on time allocation was analyzed to test if females sensed the quality of the host. For this purpose, we used the behavioral data of the pre-experience phase of the two-hour exposure group. These females had access to a host of low, medium or high quality for two hours. Time allocation was influenced by the quality of the current host. Females spent a significantly higher proportion of the two-hour period on high quality hosts compared to medium or low quality hosts (GLM with Binomial error distribution, $N = 69$, $F_{2,66} = 107.51$, $P < 0.001$) (Figure 6.3).

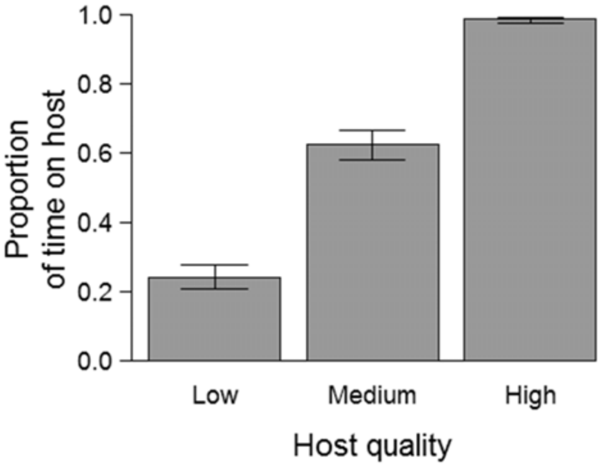

Figure 6.3 Influence of current host quality on time allocation decisions of *Nasonia vitripennis* females, estimates (± SE) derived from the statistical model, the proportion of the two-hour foraging time females spent on the host increased with increasing host quality

The influence of the average host value (manipulated through previous host quality) and of the different 'host examination modes' on time allocation was analyzed using behavioral data of the experimental phase. Here females with different experiences (only drilling into or two-hour exposure to a low, medium or high quality host) had access to the same kind of host until they abandoned that host. The estimated initial egg load was calculated using the above egg maturation rate, size of female and the time she spent in the experimental phase. As can be seen from the negative values for initial egg load (Figure 6.4), this probably slightly overestimated egg maturation and therefore underestimated initial egg load, but the error should be the same for all females (see discussion).

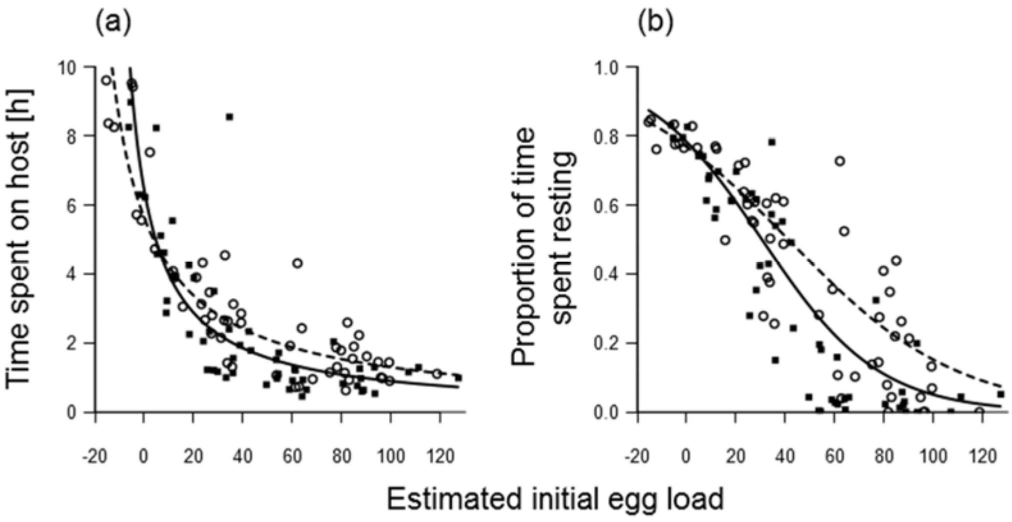

Figure 6.4 Time allocation decisions of *Nasonia vitripennis* females as a function of their egg load and their different experiences on the previous host (raw data and regression lines derived from the statistical models; only drilling: open circles, dashed line; two-hour exposure: filled squares, solid line) **a)** total time females spent on the host until abandoning it **b)** proportion of their total time allocation females spent resting on the host

Time allocation was influenced by 'host examination mode' on the previous host but not by the quality of the previous host (Table 6.2). There was a strong negative relationship between initial egg load and time spent on the host and egg load even influenced how 'host examination mode' affected time spent on the host (Table 6.2, significant interaction term: 'initial egg load * host examination mode'). At low egg loads females spent less time on the host after a drilling experience on the previous host than after an oviposition opportunity, the pattern was reversed at high egg loads (Figure 6.4a).

Table 6.2 Results of the statistical analysis of patch time allocation during the experimental phase, GLM with Gamma error distribution, N = 107

Variable	d.f.	Residual d.f.	F	P
host examination mode * host quality * initial egg load	2	95	0.2836	0.754
host examination mode * host quality	2	99	0.7968	0.454
initial egg load * host quality	2	97	0.0656	0.937
initial egg load * host examination mode	1	103	9.6671	0.002
host quality	2	101	0.5010	0.607
host examination mode	1	103	0.1250	0.724
initial egg load	1	103	190.7995	< 0.001

To test whether the long times allocated to the host resulted from high resting times or only from generally slower behavior, the proportion of time on the host the females spent resting was analyzed. There was a strong negative relationship between egg load and proportion of time spent resting (Table 6.3). With decreasing egg loads females spent an increasingly higher proportion of the time on the host resting (Figure 6.4b).

Table 6.3 Results of the statistical analysis of proportion of resting time during the experimental phase, GLM with binomial error distribution corrected for overdispersion, N = 107

Variable	d.f.	Residual d.f.	F	P
host examination mode * host quality * initial egg load	2	95	1.8559	0.162
host examination mode * host quality	2	97	0.1308	0.878
initial egg load * host quality	2	99	0.3648	0.695
initial egg load * host examination mode	1	103	7.8656	0.006
host quality	2	101	2.0356	0.136
host examination mode	1	103	3.4743	0.065
initial egg load	1	103	326.0764	< 0.001

6.5 Discussion

Egg maturation rate and Lack Clutch Size

Despite the fact that egg limitation is still controversially debated from an evolutionary point of view (Rosenheim 1996; Sevenster, Ellers & Driessen 1998; Ellers, Sevenster & Driessen 2000; Rosenheim 2011), it has been recognized to occur in several species where it influences parasitoid lifetime fitness gain (Heimpel, Mangel & Rosenheim 1998; Heimpel & Rosenheim 1998; Casas *et al.* 2000; Segoli & Rosenheim 2013). Along the same line, egg load has been found to influence egg laying decisions in insect parasitoids (Rosenheim & Rosen 1991; Minkenberg, Tatar & Rosenheim 1992; van Randen & Roitberg 1996; Koppik, Thiel & Hoffmeister 2014; Migani, Ekesi & Hoffmeister 2014).

Our study demonstrates transient egg limitation in *Nasonia vitripennis*, a highly synovigenic gregarious parasitoid. Depending on the size of a female, egg maturation rate varies between 10 and 100 eggs per day (Figure 6.1). The lower extreme might be rather artificial, since clutch size decisions of females should avoid severe competition between offspring (Koppik, Thiel & Hoffmeister 2014) and therefore very small female offspring with very low egg maturation rates should seldom be produced. Yet, the very high egg maturation rates found in our experiment may be uncommon as well. These rates are not only a result of female size but also of the (artificially) high host encounter (and therefore host feeding) rate (Edwards 1954a; Richard & Casas 2012). Egg maturation rates in the field might therefore take values in between those two extremes. Assuming an average egg maturation rate of 45 eggs per day Lack Clutch Size estimations in our and a previous study (Charnov & Skinner 1984) suggest that a patch containing 2–4 suitable hosts might already exceed the daily egg supply of a female. In our study the Lack Clutch Size was approximately 32 eggs per host (*Calliphora vomitoria*, 52.5–57.5 mg) (Figure 6.2d). A previous study by Charnov and Skinner (1984) estimates the Lack Clutch Size for *N. vitripennis* on *Phormia regina* (Diptera, Calliphoridae) hosts of varying size between 10 and 25 eggs. In line with the assumption in the Charnov and Skinner study (1984), we did not estimate clutch size dependent mortality to obtain the Lack Clutch Size, since a previous study showed that mortality is negligible in live host of *C. vomitoria* (Koppik, Thiel & Hoffmeister 2014). We only estimated clutch size dependent female offspring size (Figure 6.2a), which in turn affects lifetime fecundity of females (Figure 6.2c). In contrast to a previous study on *N. vitripennis* (Charnov & Skinner 1984) we also included sex ratio of the clutch, since the proportion of females in a clutch increased with increasing clutch size (Figure 6.2b). This resulted in a higher Lack Clutch Size estimate than using the clutch size independent average proportion of females in a clutch (Figure 6.2d). The Lack Clutch Size is possibly lower in the field (Hardy, Griffiths & Godfray 1992), because of stronger effects of size on mortality in the field compared to the laboratory (West, Flanagan & Godfray 1996). Additionally size might also affect other important factors like host finding ability (Visser 1994; Bezemer & Mills 2003). We conclude that our Lack Clutch Size estimate sets an upper limit rather than representing the average clutch size that would be obtained from the field. Yet, even if clutch sizes would be smaller than suggested by our study, field studies have demonstrated that host densities in patches can largely exceed 2–4 hosts (Grillenberger *et al.* 2008; Schurmann *et al.* 2009), making it very likely that the general conclusion from our laboratory data – that females might face transient egg limitation – still holds.

Time allocation

Egg load, and in turn the probability of becoming egg limited, has an impact on female oviposition decisions in many insects (Minkenberg, Tatar & Rosenheim 1992). The influence of egg load on clutch size decisions is well studied (Godfray, Partridge & Harvey 1991). In many, though not all species (e.g. Thiel & Hoffmeister 2006), the fewer eggs a female has available the fewer eggs she lays on a given host or patch (Odendaal & Rausher 1990; Koppik, Thiel & Hoffmeister 2014; Migani, Ekesi & Hoffmeister 2014). Models usually predict that females should lay a certain fraction of their current egg supply (when arriving on a patch) and then leave (Iwasa, Suzuki & Matsuda 1984; Mangel, Rosenheim & Adler 1994). The model of Charnov and Skinner (1988) predicts that females optimizing their fitness per unit of time should lay their complete egg complement (as long as it does not exceed the Lack Clutch Size) and immediately leave to search for new hosts, as long as travel time between patches is constant within their habitat. For stochastic travel times varying between patches, their model predicts females to stay on the patch to mature further eggs before departing under certain circumstances (Charnov & Skinner 1988). They hypothesize that after short travel times, and therefore at very low egg loads, females should stay on the host to mature and lay further eggs (up to a threshold time).

This threshold time should depend on the average travel time, the average host value in the habitat and on the value of the current host. We tested whether *N. vitripennis* stays on the host at lower egg loads and whether this depends on perceived habitat quality. Since females continuously mature eggs, we estimated initial egg load by subtracting the amount of eggs that may have been matured during the experiment from the eggs laid plus the mature eggs that remained in the ovaries. Our calculations resulted in some negative values for initial egg loads (Figure 6.4), most likely reflecting that egg maturation rate in our time allocation experiment was lower than expected from the egg maturation function. The egg maturation function was derived from females that had constant access to four fresh hosts per day, while females in the time allocation experiment only encountered one host per day, which probably resulted in fewer host feeding events and therefore in a lower egg maturation rate. Though the underestimation of initial egg load is only apparent at very low egg loads, it should have occurred across all females. Thus, the initial egg load estimate should not have led to any systematical error in the analyses.

To test whether average host value influenced the time allocation of *N. vitripennis* females, we manipulated the quality of the host within the pre-experience phase. Time allocation of females in the pre-experience phase clearly showed an effect of the quality of the host (Figure 6.3). This confirms that females sensed the quality of the host. However, previous host quality (and therefore average host value in the habitat) did not significantly influence subsequent time allocation of females. Time spent on the host was mainly influenced by the initial egg load of females, with very long times allocated to the host at low egg loads (Figure 6.4a). 'Host examination mode' also influenced the time allocation of females; a previous drilling experience led to a higher time allocation. A previous study demonstrated that females laid higher clutches after they had a drilling experience on the host compared to females that were exposed to a host for a two-hour period (chapter 5). Assuming that clutch size can be taken as a proxy for the habitat quality estimation of parasitoid females, this leads to the conclusion that females with only a previous drilling experience had a lower expectation of habitat quality than females that had a two-hour experience on the host. Thus, habitat quality estimation may have also influenced the time allocation of females, leading to longer times on the host after a previous drilling experience compared to a previous two-hour experience. Though the average host value did not influence time allocation, previous host examination mode (and therefore probably a female's habitat quality estimate) changed time allocation decisions of females. This is in accordance with the predictions by Charnov and Skinner (1988) who state that the threshold time of females should be higher at lower average host values or larger patch distances (= lower habitat quality).

According to the predictions by Charnov and Skinner (1988) high time allocation on a host should arise because females wait on the host to mature eggs, but low egg loads could also slow down the behavior of females in general. Therefore, we did not test the influence of previous experience and egg load on total resting time but on the proportion of resting time. The proportion would remain constant given that females running out of eggs show generally slower behavior. In contrast, we found a sharp increase in the proportion of time spent resting with decreasing egg loads (Figure 6.4b). This demonstrates that females at low egg loads invested more time in resting, most likely waiting to mature further eggs. The proportion of resting was again related to how the wasps had perceived the average habitat quality (through 'host examination mode' not previous host quality, Table 6.3), confirming the hypothesis of Charnov and Skinner (Charnov & Skinner 1988). Yet, our data did not reveal a common threshold time, at which females left the host. There was a continuous increase in time allocation with decreasing egg loads (Figure 6.4a), suggesting that there is no threshold in waiting time in *N. vitripennis*.

In conclusion, we demonstrated that the synovigenic parasitoid *Nasonia vitripennis* might be constrained by its daily egg maturation rate thus facing transient egg limitation. We further showed that females adapted a strategy to cope with this egg limitation by remaining on the host, most likely to mature and lay further eggs. We suggest that theoretical models of optimal clutch size in gregarious insects might also consider resting times of females, as this in turn might influence the number of eggs a female has available for oviposition.

6.6 Acknowledgements

This study was funded by the Central Research Development Fund, University of Bremen, grant 02/125/10 to AT.

7 General discussion and conclusions

Adapting foraging behavior to variable environments often requires the acquisition, storage and use of information, coming from various sources, (McNamara & Houston 1985; Ydenberg, Brown & Stephens 2007). When it comes to analyzing information processing in foraging behavior, parasitoid females have proven to be excellent study systems (see Wajnberg 2006 for a review), since their foraging success is closely linked to their reproductive success (Godfray 1994). Female parasitoids have to efficiently detect and exploit their hosts in order to maximize their lifetime fitness gain. By now, behavioral ecologists have developed sophisticated models on how a foraging animal may behave in order to maximize its lifetime fitness gain (e.g. Iwasa, Suzuki & Matsuda 1984; Mangel, Rosenheim & Adler 1994). Those models show the importance of state-dependent (Iwasa, Suzuki & Matsuda 1984; Mangel 1989; Nonacs 2001) as well as information based decision making (McNamara 1982; McNamara & Houston 1985; Roitberg, Zimmermann & Hoffmeister 2010) under a variety of ecological conditions. While the behavior of many species matches the qualitative predictions of those models quite well (e.g. Rosenheim & Rosen 1991; Visser, van Alphen & Nell 1992; Thiel & Hoffmeister 2004; Tentelier, Desouhant & Fauvergue 2006; Louapre *et al.* 2011), the behavior of other species showed a lack of such a match (see Wajnberg 2006 for a review; Tentelier, Lacroix & Fauvergue 2009). Such a mismatch might indicate constraints acting on the behavior of an animal, such as the physiological state of an individual (Nonacs 2001) or the inability to perceive or retain certain information. Therefore, the role of information use and its possible limitations are of major interest to understand how organisms adjust their behavior in an adaptive way (Krebs & Davies 1997; Dukas 1998a). The present study aimed at investigating the role of information use and learning – as one mechanism behind information use – during foraging in the parasitoid *Nasonia vitripennis*. The presented experiments were conducted to 1) investigate and disentangle the influence of information and physiological state on decision making, 2) examine the habitat information that is perceived by the female and 3) elucidate, which of these information are stored and used in later foraging decisions. Aspects of information use during foraging in *N. vitripennis* were studied in host finding through associative learning and in egg and time allocation decisions under variable habitat qualities.

7.1 State-dependent decision making during foraging

Many models used to predict female oviposition strategies use a rate-maximization approach (Charnov 1976) that is applicable to parasitoids that are time limited in their lifetime reproductive success (Wajnberg 2006). The dynamic state-variable approach allows to include the physiological state of an individual (Mangel 1989) such that they are applicable to parasitoids where lifetime reproductive success may be limited by the number of eggs available for oviposition (Rosenheim 1999). There is an ongoing debate on the evolution of egg and time limitation (Rosenheim 1996; Sevenster, Ellers & Driessen 1998; Ellers, Sevenster & Driessen 2000; Rosenheim 2011), but both have been recognized as costs of reproduction in parasitoid females (Rosenheim & Rosen 1991; Rosenheim 1999; Diaz-Fleischer & Aluja 2003). Thus, one question that needs to be addressed in order to understand female foraging decisions is whether a female is mainly egg or time limited. Clutch size decisions in *Nasonia vitripennis* females were strongly dependent on their current egg load with smaller clutches being laid at lower egg loads (Figure 3.4 and 5.3). This indicates that avoiding egg depletion is a main factor driving foraging decisions in *N. vitripennis* females. Estimated egg maturation rate in *N. vitripennis* females was on average 45 eggs per day (Figure 6.1) and the single host optimum (Lack Clutch Size) of a common host (*Calliphora vomitoria*, 52.5-57.5 mg) was approximately 32 eggs per host (Figure 6.2d). Field studies report host densities of up to 148 hosts per nest box (Grillenberger *et al.* 2008; Schurmann *et al.* 2009) and 127 per mouse carcass (Schurmann *et al.* 2009), thus *N. vitripennis* females can easily face transient egg limitation. In the laboratory we also found that females lived on average 3 days longer than they laid eggs (chapter 6.4: Egg maturation rate and longevity), one possible explanation might be terminal egg limitation, further supporting the assumption that *N. vitripennis* can be limited in its lifetime reproductive success by the number of eggs available for oviposition. Females were not dissected after death to check for remaining eggs in the ovaries. Therefore, it cannot be excluded that females still had eggs at the end of their lives but did not lay these eggs for other reasons such as abrasion of the ovipositor. Nevertheless, the present study shows that *N. vitripennis* can become (transiently) egg limited. Thus, the reduction of clutch size in response to low egg loads, low current host quality and high quality habitats seems adaptive to maximize lifetime fitness in *N. vitripennis* females

(Iwasa, Suzuki & Matsuda 1984; Skinner 1985; Mangel 1987; Mangel, Rosenheim & Adler 1994; Mangel & Heimpel 1998).

A reduction of clutch size in response to low egg loads suggests a corresponding reduction of time allocation, since laying fewer eggs should take up less time. In contrast, females of *N. vitripennis* showed a strong increase in time allocation in response to low egg loads (Figure 6.4a). This is in line with a theoretical model suggesting that females with low egg loads may remain on a host to mature and lay further eggs (Charnov & Skinner 1988). In accordance with this prediction the time allocation of females with low egg loads consisted of more than 80 % resting time, while females with very high egg loads spent less than 10 % of their time resting (Figure 6.4b). This phenomenon has seldom been shown before and may be a behavioral adaptation of *N. vitripennis* to transient egg limitation. With very small clutch sizes the increase in fitness per egg is still very high (Figure 6.2d). With stochastic host distributions the next host encounter might be unsure while fitness gain on the current host is still high such that staying on the host to mature and lay further eggs might be the best strategy. Additionally, this behavior allows for repeated host feeding, which is essential for further egg maturation in *N. vitripennis* (Edwards 1954a; Richard & Casas 2012). The present study shows an interesting behavioral adaptation to transient egg limitation in parasitoid females and suggests further research to confirm that females not only remain on the host but engage in further oviposition bouts. This in turn could influence models on clutch size decisions, since females may lay more eggs on a host, than they had available upon arrival.

In summary, egg state of a female had a strong impact on foraging decision making in *N. vitripennis*, showing behavioral adaptations to transient egg limitation. Therefore, the analysis of foraging decisions in *N. vitripennis* should include egg load as this may otherwise be an overlooked underlying state-dependent mechanism in behavioral adaptations to habitat parameters.

7.2 Information use during foraging

One important aspect of information use in foraging decisions is the perception of the forager (Sherry & Mitchell 2007). Reduced offspring numbers from low quality hosts have been reported for *Nasonia vitripennis*, suggesting that females perceive the quality of the host they parasitize (Wylie 1963; Wylie 1967; King & Rafai 1970; Werren 1984; Rivers & Denlinger 1995a; Rivers 1996; Shuker *et al.* 2005). To confirm that these reduced offspring numbers result from a female's clutch size decision (and thus from her perception of host quality) and not from increased offspring mortality on low quality hosts, offspring mortality on a variety of host qualities has been investigated (chapter 3). With the exception of dead *Calliphora vomitoria* hosts, offspring mortality was negligible on all host qualities tested (Figure 3.2). Thus, females adjusted the number of eggs they laid in response to the quality of the host they perceived: clutch sizes were reduced on small, dead and previously parasitized hosts (Figure 3.4). This influence of the quality of the current host on clutch size and on time allocation was further confirmed by a later experiment (Figure 5.2 and 6.3). Hence, time and egg allocation decisions demonstrated that females perceived this habitat information and used it in current foraging decisions.

One challenge when trying to understand the behavioral response to habitat parameters is to disentangle the influence of physiological and information state (Rosenheim & Rosen 1991), as these are often manipulated together. In *N. vitripennis* the egg state of a female has a great impact on her foraging decisions (Figure 3.4, 5.2 and 6.3) and egg load may change with different experiences. Therefore, it would be desirable to manipulate the information state about habitat parameters in females without manipulating their egg state. In associative learning of host related chemical cues the information state (where to find hosts) of a female can be changed without necessarily changing her egg state. Females of *N. vitripennis* that have successfully drilled into a host (full insertion of the ovipositor into the host puparium) in the presence of a novel odor, associate this odor with the presence of a host later on (Schurmann *et al.* 2009) without any changes in their egg state. The learning experiments within the present study (chapter 4) revealed that in this strain (HVRx, van de Zande *et al.* 2014) associative learning occurs as soon as females have begun Tapping behavior on the host (Figure 4.5). Learning and subsequent long term memory formation was elicited through a successful Drilling experience in the presence of a novel odor (Figure 4.2). This shows that females of *N. vitripennis* perceived a host encounter as such latest during Tapping. Drilling should additionally enable females to examine the content of the host puparium, which probably already changes their information state about the quality of the encountered host. In associative learning, though, quality of the host did not influence the memory formation in *N. vitripennis* females. Females formed long term memory and showed the same rate of memory retention after having drilled into a live or a dead *Lucilia sericata* host (Figure 4.7). This is in line with other studies on associative learning in *N. vitripennis* where host quality (= reward quality) did not influence learning and memory retention (Hoedjes 2014).

In summary, *N. vitripennis* females perceived the quality of the host they parasitized and used this information for current clutch size and time allocation decisions. Host encounters were perceived as such latest during Tapping and the information on a host encounter was used in subsequent host finding (through associative learning). Host quality seemed not to influence associative learning of host related odor cues.

The question whether this information on host quality and host encounters is used in subsequent foraging decisions was addressed in chapters 5 and 6. Females perceive a host encounter through a successful drilling event into a host (chapter 4) and this should also enable them to sense the quality of the host (King & Rafai 1970), therefore it was hypothesized that a female’s information state about habitat quality could also be altered through a drilling experience into different quality hosts (chapter 5). This would change her information state without major changes in her physiological state (especially egg load). Therefore, an analysis of clutch size and time allocation decisions in *N. vitripennis* females was conducted, using two different ‘host examination modes’ on hosts of different qualities. These ‘host examination modes’ should either only change the information state of a female (only drilling) or should also change her egg state (two hour exposure). Female clutch size and time allocation decisions have been corrected for egg load (by including egg load into the statistical models), since this might be an underlying state-dependent mechanism influencing a female’s decisions (see above). Surprisingly, females did not significantly change their subsequent clutch size or time allocation decisions in response to the quality of the previous host, irrespective of the ‘host examination mode’ (Table 5.1 and 6.2). Additionally, within the females that had laid eggs on the previous host there was no significant influence of the number of eggs deposited on that host (which could be a measure of host quality) on the subsequent clutch size decision (Table 5.3). This is in contrast to many other studies where quality of the previous patch (see Wajnberg 2006 for a review) or eggs laid previously (Froissart *et al.* 2012) had an impact on current foraging decisions. Though, *N. vitripennis* females perceived the quality of the previous host, they did not use the information for subsequent foraging decisions. This resembles the results from associative learning experiments, where host quality did not influence learning or memory retention in *N. vitripennis* (chapter 4). An explanation for this may be that the quality of one host encountered is of low predictive value concerning the quality of hosts that may be encountered in the future, which is quite likely since host patches often contain a mixture of hosts in the field (Peters 2010; Daoust *et al.* 2012). This would make host quality an unimportant information for future host finding and foraging decisions in *N. vitripennis*.

While previous host quality had no impact on subsequent foraging decisions within both ‘host examination modes’, the ‘host examination mode’ itself significantly altered clutch size and time allocation decisions (Figure 5.3 and 6.4). At the same egg load, clutch size and time allocation was higher for females that were only allowed to drill into the host compared to females that were exposed to a host for two hours including the possibility to host feed and lay eggs. The change in clutch size was not induced by egg laying, which makes an information and not state-dependent mechanism most likely. The differences may indicate that females simply did not perceive the drilling experience as a host encounter, but the results of the associative learning experiments (Figure 4.2) are contradictory to this explanation. Habituation to the host odor may account for the lowered time allocation after a previous two-hour exposure to a host, but the high time allocation in response to low egg loads (Figure 6.4a) exclude a simple habituation mechanism in time allocation decisions. Lower exploitation of a host is expected for higher habitat quality estimates (Charnov 1976), lower quality of the current host (Skinner 1985) or lower egg load (Iwasa, Suzuki & Matsuda 1984). Female foraging decisions were measured on hosts of the same quality and differences between different ‘host examination modes’ appeared at the same egg load (Figure 5.3 and 6.4). Therefore, it can be hypothesized that females with the ‘two-hour exposure’ experience (that laid smaller clutches and spent less time on the subsequent host) had a higher habitat quality estimate. An explanation for this might be that a longer contact with the host provided the females with more information about the suitability of that host making the experience more reliable. Only drilling, thus the withdrawal of the ovipositor immediately after insertion, mostly occurs when a female encounters a host completely unsuitable for parasitization. Therefore, information based on the host examination might have signaled an encounter of a suitable host to the female, while the (artificial) termination of the experience after drilling might have indicated the encounter of an unsuitable host. Thus, without further contact and probably information gathering through host feeding, the encounter with a suitable host may remain unsure/unreliable for the female. This difference in reliability might have led to differences in habitat quality estimates. Cue reliability might also account for differences in memory retention between females that were only allowed to drill into the host during associative learning and females that were also allowed to host feed (Figure 4.2). Though both conditioning types led to the formation of long term memory for the presented odor, host feeding led to a higher memory retention rate. These parallels suggest that initial information processing in habitat quality estimation and associative learning of host related cues may use the same pathway in *N. vitripennis*.

The present study shows that females of *N. vitripennis* not only made state-dependent foraging decisions but also included information about their habitat in their host finding and foraging decision making processes. Though information about host encounter and host quality was perceived by the female, the latter was not used in subsequent decisions. The study also points out an interesting parallel in information use in host finding through associative learning and habitat quality estimations that may indicate the use of the same pathway in initial information processing in these two processes.

7.3 Learning and memory formation during foraging

Nasonia vitripennis shows the ability of associative learning of host related odors (Schurmann *et al.* 2009; Hoedjes *et al.* 2012; Schurmann *et al.* 2012), which may facilitate their host finding success as has been shown for other parasitoid species (Papaj & Vet 1990). The present study demonstrates that females learned to associate a novel odor with the presence of a host and stored this information in different types of memories (chapter 4). Memory retention was influenced by the intensity and number of the host experiences during learning (Figure 4.2). These findings are in line with other studies on associative learning in *N. vitripennis* (Schurmann *et al.* 2009; Schurmann *et al.* 2012; Hoedjes & Smid 2014). In the present study memory retention was influenced in two ways: 1. The type of memory formed after conditioning was different for different host experience intensities. While a Tapping experience elicited the formation of medium term memory (Figure 4.5), more intense host experiences elicited the formation of long term memory (Figure 4.2). 2. Within the host experiences that elicited long term memory formation the rate of memory retention was influenced by intensity and number of host experiences. Increasing the intensity or number of host encounters increased the memory retention rate (Figure 4.2). This is most likely due to the fact that the intensity of the host experience may have influenced the reliability of information about an encounter with a suitable host for the females. The more reliable an information is, with regard to future conditions, the more likely it should be learned and stored in longer lasting, more stable memories (Eliassen *et al.* 2009). The reliability of an information does not only depend on the reliability of an individual's perception, but also depends on the predictability of future conditions (within generation persistence) (Stephens 1991). The possible influence of habitat predictability on learning strategies has been demonstrated in two closely related parasitoid wasps, *Cotesia glomerata* and *Cotesia rubecula* (Hymenoptera: Braconidae). While *C. glomerata* forms a stable, consolidated memory lasting, up to 5 days, after a single oviposition experience in the presence of an odor, *C. rubecula* only forms a shorter memory that starts to wane after 1 day (Bleeker *et al.* 2006). Long term memory formation only required a single learning trial in *C. glomerata* but three spaced trainings in *C. rubecula* (Smid *et al.* 2007). These differences in memory formation go along with differences in the spatial distributions of their hosts. *C. glomerata* prefers *Pieris brassicae* as a host, which lays its eggs in large clutches on one host plant, thus finding one host in the presence of an odor has a high predictive value for future host encounters (Bleeker *et al.* 2006). *C. rubecula* on the other hand parasitizes *Pieris rapae*, which lays single eggs preferably on isolated plants of different plant species, thus in *C. rubecula* finding a host associated with a certain odor has a much lower predictive value for future host encounters (Bleeker *et al.* 2006). This suggests that species-specific learning and memory formation strategies in these two species are adapted to their different ecologies.

While associative learning and interspecific differences in learning have already been extensively studied in parasitoids (see Hoedjes *et al.* 2011 for a review) and in particular in *N. vitripennis* (Schurmann *et al.* 2009; Hoedjes *et al.* 2012; Schurmann *et al.* 2012; Hoedjes & Smid 2014), intraspecific variability has been investigated to a lesser extent (but see Kruidhof *et al.* 2012; Thiel, Schlake & Kosior 2013). Using a genetically variable line (HVRx, van de Zande *et al.* 2014) and an iso-female line (AsymCx, Werren *et al.* 2010), the sources of intra-specific variability in learning behavior in *N. vitripennis* was investigated. The comparison of variance in memory retention between the genetically diverse strain and the iso-female line revealed that at least part of the variance is due to genetic variability in the genetically diverse strain. A closer examination of the variance within the genetically diverse strain demonstrated that females did not differ in their memory duration, but in the intensity of the host experience that was needed to elicit associative learning (Figure 4.3 and 4.4). With increasing intensity of the host experience during conditioning, an increasing part of the females seemed to have learned to associate the odor with the presence of a host, which decreased the variance in memory retention (Figure 4.3). Individual females of *N. vitripennis* seem to differ in the reliability that a cue needs to have in order to elicit learning, and the results from the iso-female line suggest that this variability is at least partly due to genetic variability. This suggests that females may consistently differ in their information use in host finding. Genetic variability in learning behavior may have been maintained due to the variable micro-habitats *N. vitripennis* inhabits. Hosts of *N. vitripennis* can be found in birds' nests as well as in various animal carcasses (Abraham 1985; Voss, Spafford & Dadour 2009). While carcasses might provide a female with enough hosts for her entire lifetime (even relatively small mouse carcasses contain 21–127 hosts (Schurmann *et al.* 2009)), a female might have to visit several nest boxes to find enough suitable hosts (30 % of the investigated nest boxes in a study by Schurmann et al. (2009) contained only a single host). This gives rise to new questions regarding the adaptations to ecological differences within a species that may lead to different learning strategies in parasitoids. Intraspecific differences also open up the possibility to study trade-offs between learning and life history traits in parasitoids complementing the thorough work that has already been conducted in fruit flies (see Kawecki 2010 for a review).

Parasitoid females have been shown to use information about the reward value storing the learned information in different memories for different host qualities (Kruidhof *et al.* 2012). There was no such influence of host quality within the present study (Figure 4.7). Though females perceived the death of a host and reduced their clutch size in response to dead hosts (Figure 3.4), memory formation did not differ for females receiving a dead host compared to females that received a live host as a reward in associative learning experiments (see 4.7). This result is supported by another study that demonstrated that even different host species do not influence memory retention (Hoedjes 2014). By now, studies suggest that reward value has no influence on learning of host associated odors in *N. vitripennis*, such that this information has no influence on host finding decisions.

The experiments on associative learning of host related odors demonstrated that females used information about host encounters but not about host quality in host finding. Additionally, the experiments revealed considerable differences between females in associative learning. This gives rise to the question, whether there are also consistent differences between females in other aspects of information use during foraging, and which mechanisms lead to the maintenance of these differences in natural populations of *Nasonia vitripennis*.

7.4 Conclusions

Three major conclusions can be drawn from the present study:

1. The results strongly suggest that females of *Nasonia vitripennis* face transient egg limitation within the range of natural host encounter rates and show behavioral adaptations to this condition. Foraging decisions on egg and time allocation were highly dependent on physiological state, i.e. the current egg supply of a female. Time allocation in *N. vitripennis* may be a special behavioral adaptation to transient egg limitation, as females with low egg loads remained on the host after parasitization rather than leaving immediately to search for a new host. This suggests that models on clutch size decisions might need to consider additional egg maturation to predict final clutch size on a host in egg limited females.

2. Foraging decisions in *N. vitripennis* include information about host quality and host encounters, but information use differs for current and subsequent decisions. Time allocation and clutch size decisions on a given host were affected by the quality of the current host. Clutch size, time allocation and host finding decisions were also affected by previous host encounters. Yet, they were not affected by previous host quality. This indicates an adaptation of information use to the predictive value of the information, as host patches may contain various hosts of different qualities. The parallels seen in host finding through associative learning and information use in clutch size decisions also indicate a common pathway in initial information processing.

3. Associative learning and subsequent memory formation shows considerable intraspecific variability, which might be maintained due to differences in the micro-habitats that are inhabited by *N. vitripennis*. Yet, this remains to be tested in future research on the mechanisms that preserve variability in learning behavior within natural populations of parasitoids.

8 Appendix

8.1 References

Abraham, R. (1985) *Nasonia vitripennis* an insect from birds nests [Hymenoptera, Chalcidoidea, Pteromalidae]. *Entomologia Generalis,* **10,** 121-124.

Alghamdi, A., Raine, N.E., Rosato, E. & Mallon, E.B. (2009) No evidence for an evolutionary trade-off between learning and immunity in a social insect. *Biology Letters,* **5,** 55-57.

Baeder, J.M. & King, B.H. (2004) Associative learning of color by males of the parasitoid wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae). *Journal of Insect Behavior,* **17,** 201-213.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2014) lme4: Linear mixed-effects models using Eigen and S4. *R package version 1.1-6*.

Bezemer, T.M. & Mills, N.J. (2003) Clutch size decisions of a gregarious parasitoid under laboratory and field conditions. *Animal Behaviour,* **66,** 1119-1128.

Bleeker, M.A.K., Smid, H.M., Steidle, J.L.M., Kruidhof, H.M., Van Loon, J.J.A. & Vet, L.E.M. (2006) Differences in memory dynamics between two closely related parasitoid wasp species. *Animal Behaviour,* **71,** 1343-1350.

Boivin, G., Fauvergue, X. & Wajnberg, E. (2004) Optimal patch residence time in egg parasitoids: innate versus learned estimate of patch quality. *Oecologia,* **138,** 640-647.

Brundage, A., Bros, S. & Honda, J.Y. (2011) Seasonal and habitat abundance and distribution of some forensically important blow flies (Diptera: Calliphoridae) in Central California. *Forensic Science International,* **212,** 115-120.

Burger, J.M.S., Kolss, M., Pont, J. & Kawecki, T.J. (2008) Learning ability and longevity: A symmetrical evolutionary trade-off in *Drosophila*. *Evolution,* **62,** 1294-1304.

Burns, J.G., Foucaud, J. & Mery, F. (2011) Costs of memory: lessons from 'mini' brains. *Proceedings of the Royal Society B-Biological Sciences,* **278,** 923-929.

Burton-Chellew, M.N., Koevoets, T., Grillenberger, B.K., Sykes, E.M., Underwood, S.L., Bijlsma, R., Gadau, J., van de Zande, L., Beukeboom, L.W., West, S.A. & Shuker, D.M. (2008) Facultative sex ratio adjustment in natural populations of wasps: cues of local mate competition and the precision of adaptation. *American Naturalist,* **172,** 393-404.

Casas, J., Nisbet, R.M., Swarbrick, S. & Murdoch, W.W. (2000) Eggload dynamics and oviposition rate in a wild population of a parasitic wasp. *Journal of Animal Ecology,* **69,** 185-193.

Casas, J., Vannier, F., Mandon, N., Delbecque, J.P., Giron, D. & Monge, J.P. (2009) Mitigation of egg limitation in parasitoids: immediate hormonal response and enhanced oogenesis after host use. *Ecology,* **90,** 537-545.

Castro, C.P.E., Serrano, A., Da Silva, P.M. & Garcia, M.D. (2012) Carrion flies of forensic interest: a study of seasonal community composition and succession in Lisbon, Portugal. *Medical and Veterinary Entomology,* **26,** 417-431.

Charnov, E.L. (1976) Optimal foraging, Marginal Value Theorem. *Theoretical Population Biology,* **9,** 129-136.

Charnov, E.L. & Skinner, S.W. (1984) Evolution of host selection and clutch size in parasitoid wasps. *Florida Entomologist,* **67,** 5-21.

Charnov, E.L. & Skinner, S.W. (1988) Clutch size in parasitoids: the egg production rate as a constraint. *Evolutionary Ecology,* **2,** 167-174.

Cole, Ella F., Morand-Ferron, J., Hinks, Amy E. & Quinn, John L. (2012) Cognitive ability influences reproductive life history variation in the wild. *Current Biology,* **22,** 1808-1812.

Dall, S.R.X., Giraldeau, L.A., Olsson, O., McNamara, J.M. & Stephens, D.W. (2005) Information and its use by animals in evolutionary ecology. *Trends in Ecology & Evolution,* **20,** 187-193.

Danci, A., Hrabar, M., Ikoma, S., Schaefer, P.W. & Gries, G. (2013) Learning provides mating opportunities for males of a parasitoid wasp. *Entomologia Experimentalis Et Applicata,* **149,** 229-240.

Danneels, E.L., Rivers, D.B. & De Graaf, D.C. (2010) Venom proteins of the parasitoid wasp *Nasonia vitripennis*: recent discovery of an untapped pharmacopee. *Toxins,* **2,** 494-516.

Daoust, S.P., Savage, J., Whitworth, T.L., Belisle, M. & Brodeur, J. (2012) Diversity and abundance of ectoparasitic blow flies *Protocalliphora* (Diptera: Calliphoridae) and their *Nasonia* (Hymenoptera: Pteromalidae) parasitoids in Tree Swallow nests within agricultural lands of Southern Quebec, Canada. *Annals of the Entomological Society of America,* **105,** 471-478.

Darling, D.C. & Werren, J.H. (1990) Biosystematics of *Nasonia* (Hymenoptera, Pteromalidae): two new species reared from birds' nests in North America. *Annals of the Entomological Society of America,* **83,** 352-370.

Davies, I. (1975) Study of effect of diet on life-span of *Nasonia-vitripennis* (Walk.) (Hymenoptera, Pteromalidae). *Journals of Gerontology,* **30,** 294-298.

Dejong, R. & Kaiser, L. (1992) Odor preference of a parasitic wasp depends on order of learning. *Experientia,* **48,** 902-904.

Desouhant, E., Debouzie, D., Ploye, H. & Menu, F. (2000) Clutch size manipulations in the chestnut weevil, *Curculio elephas*: fitness of oviposition strategies. *Oecologia,* **122,** 493-499.

Dezazzo, J. & Tully, T. (1995) Dissection of memory formation - from behavioral pharmacology to molecular genetics. *Trends in Neurosciences,* **18,** 212-218.

Diaz-Fleischer, F. & Aluja, M. (2003) Behavioural plasticity in relation to egg and time limitation: the case of two fly species in the genus *Anastrepha* (Diptera : Tephritidae). *Oikos,* **100,** 125-133.

Donaldson, J.S. & Walter, G.H. (1988) Effects of egg availability and egg maturity on the ovipositional activity of the parasitic wasp, *Coccophagus atratus*. *Physiological Entomology,* **13,** 407-417.

Dubnau, J. (2003) Neurogenetic dissection of conditioned behavior: Evolution by analogy or homology? *Journal of Neurogenetics,* **17,** 295-326.

Dukas, R. (1998a) Constraints on information processing and their effects on behavior. *Cognitive Ecology: the evolutionary ecology of information processing and decision making* (ed. R. Dukas), pp. 89-127. The University of Chicago Press, Chicago.

Dukas, R. (1998b) Evolutionary ecology of learning. *Cognitive ecology: the evolutionary ecology of information processing and decision making* (ed. R. Dukas), pp. 129-174. The University of Chicago Press, Chicago.

Dukas, R. (2008) Evolutionary Biology of Insect Learning. *Annual Review of Entomology,* **53,** 145-160.

Dukas, R. & Bernays, E.A. (2000) Learning improves growth rate in grasshoppers. *Proceedings of the National Academy of Sciences of the United States of America,* **97,** 2637-2640.

Dunlap, A.S., McLinn, C.M., MacCormick, H.A., Scott, M.E. & Kerr, B. (2009) Why some memories do not last a lifetime: dynamic long-term retrieval in changing environments. *Behavioral Ecology,* **20,** 1096-1105.

Edwards, R.L. (1954a) The effect of diet on egg maturation and resorption in *Mormoniella vitripennis* (Hymenoptera, Pteromalidae). *Quarterly Journal of Microscopical Science,* **95,** 459-468.

Edwards, R.L. (1954b) The host-finding and oviposition behaviour of *Mormoniella vitripennis* (Walker) (Hymenoptera, Pteromalidae), a parasite of muscoid flies. *Behaviour,* **7,** 88-112.

Egas, M. & Sabelis, M.W. (2001) Adaptive learning of host preference in a herbivorous arthropod. *Ecology Letters,* **4,** 190-195.

Eliassen, S., Jorgensen, C., Mangel, M. & Giske, J. (2009) Quantifying the adaptive value of learning in foraging behavior. *Am Nat,* **174,** 478-489.

Ellers, J., Sevenster, J.G. & Driessen, G. (2000) Egg load evolution in parasitoids. *American Naturalist,* **156,** 650-665.

Elzinga, J.A., Harvey, J.A. & Biere, A. (2005) Age-dependent clutch size in a koinobiont parasitoid. *Ecological Entomology,* **30,** 17-27.

Fernando, L.C.P. & Walter, G.H. (1999) Activity patterns and oviposition rates of *Aphytis lingnanensis* females, a parasitoid of California red scale *Aonidiella aurantii*: implications for successful biological control. *Ecological Entomology,* **24,** 416-425.

Ferrari, M.C.O., Brown, G.E., Bortolotti, G.R. & Chivers, D.P. (2010) Linking predator risk and uncertainty to adaptive forgetting: a theoretical framework and empirical test using tadpoles. *Proceedings of the Royal Society B-Biological Sciences,* **277,** 2205-2210.

Fox, J. & Weisberg, S. (2011) *An R companion to applied regression,* 2 edn. Sage, Los Angeles.

Froissart, L., Bernstein, C., Humblot, L., Amat, I. & Desouhant, E. (2012) Facing multiple information sources while foraging on successive patches: how does a parasitoid deal with experience? *Animal Behaviour,* **83,** 189-199.

Godfray, H.C.J. (1994) *Parasitoids. Behavioral and evolutionary ecology*. Princeton Univ. Press, Princeton, NJ.

Godfray, H.C.J., Partridge, L. & Harvey, P.H. (1991) Clutch size. *Annual Review of Ecology and Systematics,* **22,** 409-429.

Godfray, H.C.J. & Waage, J.K. (1988) Learning in parasitic wasps. *Nature,* **331,** 211-211.

Goncalves, M.F., Malheiro, R., Casal, S., Torres, L. & Pereira, J.A. (2012) Influence of fruit traits on oviposition preference of the olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), on three Portuguese olive varieties (Cobrancosa, Madural and Verdeal Transmontana). *Scientia Horticulturae,* **145,** 127-135.

Graffelman, J. (2012) Calibrate: calibration of scatterplot and biplot axes.

Grillenberger, B.K., Koevoets, T., Burton-Chellew, M.N., Sykes, E.M., Shuker, D.M., De Zande, L.V., Bijlsma, R., Gadau, J. & Beukeboom, L.W. (2008) Genetic structure of natural *Nasonia vitripennis* populations: validating assumptions of sex-ratio theory. *Molecular Ecology,* **17,** 2854-2864.

Hamilton, W.D. (1967) Extraordinary sex ratios. *Science,* **156,** 477-488.

Hardy, I.C.W., Griffiths, N.T. & Godfray, H.C.J. (1992) Clutch size in a parasitoid wasp - a manipulation experiment. *Journal of Animal Ecology,* **61,** 121-129.

Harrell, F.E.J., Dupont, C. & al., e. (2013) Hmisc: Harrell Miscellaneous. R package version 3.13-0. http://CRAN.R-project.org/package=Hmisc.

Healy, S.D., Bacon, I.E., Haggis, O., Harris, A.P. & Kelley, L.A. (2009) Explanations for variation in cognitive ability: Behavioural ecology meets comparative cognition. *Behavioural Processes,* **80,** 288-294.

Healy, S.D. & Jones, C.M. (2002) Animal learning and memory: an integration of cognition and ecology. *Zoology,* **105,** 321-327.

Heimpel, G.E., Mangel, M. & Rosenheim, J.A. (1998) Effects of time limitation and egg limitation on lifetime reproductive success of a parasitoid in the field. *American Naturalist,* **152,** 273-289.

Heimpel, G.E. & Rosenheim, J.A. (1998) Egg limitation in parasitoids: A review of the evidence and a case study. *Biological Control,* **11,** 160-168.

Heimpel, G.E., Rosenheim, J.A. & Mangel, M. (1996) Egg limitation, host quality, and dynamic behavior by a parasitoid in the field. *Ecology,* **77,** 2410-2420.

Hoedjes, K.M. (2014) Natural variation in memory formation among *Nasonia* parasitic wasps: from genes to behaviour. PhD Thesis, Wageningen University.

Hoedjes, K.M., Kruidhof, H.M., Huigens, M.E., Dicke, M., Vet, L.E.M. & Smid, H.M. (2011) Natural variation in learning rate and memory dynamics in parasitoid wasps: opportunities for converging ecology and neuroscience. *Proceedings of the Royal Society B-Biological Sciences,* **278,** 889-897.

Hoedjes, K.M. & Smid, H.M. (2014) Natural variation in long-term memory formation among *Nasonia* parasitic wasp species. *Behavioural Processes,* **105,** 40-45.

Hoedjes, K.M., Steidle, J.L.M., Werren, J.H., Vet, L.E.M. & Smid, H.M. (2012) High-throughput olfactory conditioning and memory retention test show variation in *Nasonia* parasitic wasps. *Genes, Brain and Behavior,* **11,** 879-887.

Hoffmeister, T.S. & Rohlfs, M. (2001) Aggregative egg distributions may promote species co-existence - but why do they exist? *Evolutionary Ecology Research,* **3,** 37-50.

Hoffmeister, T.S., Thiel, A., Kock, B., Babendreier, D. & Kuhlmann, U. (2000) Pre-patch experience affects the egg distribution pattern in a polyembryonic parasitoid of moth egg batches. *Ethology,* **106,** 145-157.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal,* **50,** 346-363.

Iwasa, Y., Suzuki, Y. & Matsuda, H. (1984) Theory of oviposition strategy of parasitoids. 1.Effect of mortality and limited egg number. *Theoretical Population Biology,* **26,** 205-227.

Jones, T.H. & Turner, B.D. (1987) The effect of host spatial-distribution on patterns of parasitism by *Nasonia vitripennis*. *Entomologia Experimentalis Et Applicata,* **44,** 169-175.

Kaiser, L., Perez-Maluf, R., Sandoz, J.C. & Pham-Delegue, M.H. (2003) Dynamics of odour learning in *Leptopilina boulardi*, a hymenopterous parasitoid. *Animal Behaviour,* **66,** 1077-1084.

Kapranas, A., Hardy, I.C.W., Morse, J.G. & Luck, R.F. (2011) Parasitoid developmental mortality in the field: patterns, causes and consequences for sex ratio and virginity. *Journal of Animal Ecology,* **80,** 192-203.

Kawecki, T.J. (2010) Evolutionary ecology of learning: insights from fruit flies. *Population Ecology,* **52,** 15-25.

King, B. (1992) Sex-ratios of the wasp *Nasonia vitripennis* from self-versus conspecifically-parasitized hosts - local mate competition versus host quality models. *Journal of Evolutionary Biology,* **5,** 445-455.

King, B.H. & Ellison, J.H. (2006) Resource quality affects restlessness in the parasitoid wasp *Nasonia vitripennis*. *Entomologia Experimentalis Et Applicata,* **118,** 71-76.

King, B.H. & Skinner, S.W. (1991) Proximal mechanisms of the sex-ratio and clutch size responses of the wasp *Nasonia vitripennis* to parasitized hosts. *Animal Behaviour,* **42,** 23-32.

King, P.E. & Hopkins, C.R. (1963) Length of life of sexes in *Nasonia vitripennis* (Walker) (Hymenoptera, Pteromalidae) under conditions of starvation. *Journal of Experimental Biology,* **40,** 751-761.

King, P.E. & Rafai, J. (1970) Host discrimination in a gregarious parasitoid *Nasonia vitripennis* (Walker) (Hymenoptera, Pteromalidae). *Journal of Experimental Biology,* **53,** 245-254.

Kolss, M. & Kawecki, T.J. (2008) Reduced learning ability as a consequence of evolutionary adaptation to nutritional stress in *Drosophila melanogaster*. *Ecological Entomology,* **33,** 583-588.

Koppik, M., Thiel, A. & Hoffmeister, T.S. (2014) Adaptive decision making or differential mortality: what causes offspring emergence in a gregarious parasitoid? *Entomologia Experimentalis Et Applicata,* **150,** 208-216.

Krebs, J. (1977) Optimal foraging - theory and experiment. *Nature,* **268,** 583-584.

Krebs, J.R. & Davies, N.B. (1997) Part 2: mechanisms and individual behavior. *Behavioural ecology: an evolutionary approach* (eds J.R. Krebs & N.B. Davies), pp. 15-18. Blackwell Science, Oxford.

Kruidhof, H.M., Pashalidou, F.G., Fatouros, N.E., Figueroa, I.A., Vet, L.E.M., Smid, H.M. & Huigens, M.E. (2012) Reward value determines memory consolidation in parasitic wasps. *Plos One,* **7,** e39615.

Lack, D. (1947) The significance of clutch-size. *Ibis,* **89,** 302-352.

Laverty, T.M. & Plowright, R.C. (1988) Flower handling by bumblebees - a comparison of specialists and generalists. *Animal Behaviour,* **36,** 733-740.

Lewis, W.J. & Takasu, K. (1990) Use of learned odors by a parasitic wasp in accordance with host and food needs. *Nature,* **348,** 635-636.

Liu, Y.Q., Bernstein, C. & Thiel, A. (2009) Travel duration, energetic expenditure, and patch exploitation in the parasitic wasp *Venturia canescens*. *Behavioral Ecology and Sociobiology,* **63,** 1459-1469.

Louapre, P. & Pierre, J.S. (2012) Carbon dioxide narcosis modifies the patch leaving decision of foraging parasitoids. *Animal Cognition,* **15,** 429-435.

Louapre, P., van Baaren, J., Pierre, J.S. & van Alphen, J.J.M. (2011) Information gleaned and former patch quality determine foraging behavior of parasitic wasps. *Behavioral Ecology,* **22,** 1064-1069.

Maechler, M. & al., e. (2012) sfsmisc: utilities from Seminar fuer Statistik ETH Zurich.

Mangel, M. (1987) Oviposition site selection and clutch size in insects. *Journal of Mathematical Biology,* **25,** 1-22.

Mangel, M. (1989) Evolution of host selection in parasitoids - does the state of the parasitoid matter? *American Naturalist,* **133,** 688-705.

Mangel, M. & Heimpel, G.E. (1998) Reproductive senescence and dynamic oviposition behaviour in insects. *Evolutionary Ecology,* **12,** 871-879.

Mangel, M., Rosenheim, J.A. & Adler, F.R. (1994) Clutch size, offspring performance, and intergenerational fitness. *Behavioral Ecology,* **5,** 412-417.

McNamara, J. (1982) Optimal patch use in a stochastic environment. *Theoretical Population Biology,* **21,** 269-288.

McNamara, J.M. & Houston, A.I. (1985) Optimal foraging and learning. *Journal of Theoretical Biology,* **117,** 231-249.

McNamara, J.M. & Houston, A.I. (1987) Memory and the efficient use of information. *Journal of Theoretical Biology,* **125,** 385-395.

Mery, F. & Kawecki, T.J. (2002) Experimental evolution of learning ability in fruit flies. *Proceedings of the National Academy of Sciences of the United States of America,* **99,** 14274-14279.

Mery, F. & Kawecki, T.J. (2003) A fitness cost of learning ability in *Drosophila melanogaster*. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **270,** 2465-2469.

Mery, F. & Kawecki, T.J. (2004) An operating cost of learning in *Drosophila melanogaster*. *Animal Behaviour,* **68,** 589-598.

Mery, F. & Kawecki, T.J. (2005) A cost of long-term memory in *Drosophila*. *Science,* **308,** 1148-1148.

Michaud, J.P. & Mackauer, M. (1995) Oviposition behavior of *Monoctonus paulensis* (Hymenoptera, Aphidiidae) - factors influencing reproductive allocation to hosts and host patches. *Annals of the Entomological Society of America,* **88,** 220-226.

Migani, V., Ekesi, S. & Hoffmeister, T.S. (2014) Physiology vs. environment: what drives oviposition decisions in mango fruit flies (*Bactrocera invadens* and *Ceratitis cosyra*)? *Journal of Applied Entomology,* **138,** 395-402.

Minkenberg, O., Tatar, M. & Rosenheim, J.A. (1992) Egg load as a major source of variability in insect foraging and oviposition behavior. *Oikos,* **65,** 134-142.

Molbo, D. & Parker, E.D. (1996) Mating structure and sex ratio variation in a natural population of *Nasonia vitripennis*. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **263,** 1703-1709.

Muller, C., Collatz, J., Wieland, R. & Steidle, J.L.M. (2006) Associative learning and memory duration in the parasitic wasp *Lariophagus distinguendus*. *Animal Biology,* **56,** 221-232.

Muratori, F., Boivin, G. & Hance, T. (2008) The impact of patch encounter rate on patch residence time of female parasitoids increases with patch quality. *Ecological Entomology,* **33,** 422-427.

Nelder, J.A. & Wedderburn, R.W.M. (1972) Generalized linear models. *Journal of the Royal Statistical Society Series a-General,* **135,** 370-384.

Nonacs, P. (2001) State dependent behavior and the Marginal Value Theorem. *Behavioral Ecology,* **12,** 71-83.

Odendaal, F.J. & Rausher, M.D. (1990) Egg load influences search intensity, host selectivity, and clutch size in *Battus philenor* butterflies. *Journal of Insect Behavior,* **3,** 183-193.

Oliai, S.E. & King, B.H. (2000) Associative learning in response to color in the parasitoid wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae). *Journal of Insect Behavior,* **13,** 55-69.

Outreman, Y., Le Ralec, A., Wajnberg, E. & Pierre, J.S. (2005) Effects of within- and among-patch experiences on the patch-leaving decision rules in an insect parasitoid. *Behavioral Ecology and Sociobiology,* **58,** 208-217.

Pannebakker, B.A., Trivedi, U., Blaxter, M.A., Watt, R. & Shuker, D.M. (2013) The transcriptomic basis of oviposition behaviour in the parasitoid wasp *Nasonia vitripennis*. *Plos One,* **8,** e68608.

Papaj, D.R. & Vet, L.E.M. (1990) Odor learning and foraging success in the parasitoid *Leptopilina heterotoma*. *Journal of Chemical Ecology,* **16,** 3137-3150.

Parker, G.A. & Courtney, S.P. (1984) Models of clutch size in insect oviposition. *Theoretical Population Biology,* **26,** 27-48.

Peters, R.S. (2010) Host range and offspring quantities in natural populations of *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Chalcidoidea: Pteromalidae). *Journal of Hymenoptera Research,* **19,** 128-138.

Pexton, J.J., de Boer, J.G., Heimpel, G.E., Vet, L.E.M., Whitfield, J.B. & Ode, P.J. (2009) Competition and brood reduction: testing alternative models of clutch-size evolution in parasitoids. *Behavioral Ecology,* **20,** 403-409.

Placais, P.Y. & Preat, T. (2013) To favor survival under food shortage, the brain disables costly memory. *Science,* **339,** 440-442.

Price, P.W. (1980) *Evolutionary biology of parasites*. Princeton University Press, Princeton.

Quicke, D.J.L. (1997) *Parasitic Wasps*. Chapman & Hall, London.

R Core Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

R Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raine, N.E. & Chittka, L. (2008) The correlation of learning speed and natural foraging success in bumble-bees. *Proceedings of the Royal Society B-Biological Sciences,* **275,** 803-808.

Ratcliffe, N.A. & King, P.E. (1967) The "venom" system of *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). *Proceedings of the Royal Entomological Society of London Series a-General Entomology,* **42,** 49-61.

Raychoudhury, R., Desjardins, C.A., Buellesbach, J., Loehlin, D.W., Grillenberger, B.K., Beukeboom, L., Schmitt, T. & Werren, J.H. (2010) Behavioral and genetic characteristics of a new species of *Nasonia*. *Heredity,* **104,** 278-288.

Richard, R. & Casas, J. (2012) A quantitative framework for ovarian dynamics. *Functional Ecology,* **26,** 1399-1408.

Rivero, A. & West, S.A. (2005) The costs and benefits of host feeding in parasitoids. *Animal Behaviour,* **69,** 1293-1301.

Rivers, D.B. (1996) Changes in oviposition behavior of the ectoparasitoids *Nasonia vitripennis* and *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae) when using different species of fly hosts, prior oviposition experience, and allospecific competition. *Annals of the Entomological Society of America,* **89,** 466-474.

Rivers, D.B. & Denlinger, D.L. (1994a) Developmental fate of the flesh fly, *Sarcophaga bullata*, envenomated by the pupal ectoparasitoid, *Nasonia vitripennis*. *Journal of Insect Physiology,* **40,** 121-127.

Rivers, D.B. & Denlinger, D.L. (1994b) Redirection of metabolism in the flesh fly, *Sarcophaga bullata*, following envenomation by the ectoparasitoid *Nasonia vitripennis* and correlation of metabolic effects with the diapause status of the host. *Journal of Insect Physiology,* **40,** 207-215.

Rivers, D.B. & Denlinger, D.L. (1995a) Fecundity and development of the ectoparasitic wasp *Nasonia vitripennis* are dependent on host quality. *Entomologia Experimentalis Et Applicata,* **76,** 15-24.

Rivers, D.B. & Denlinger, D.L. (1995b) Venom-induced alterations in fly lipid metabolism and its impact on larval development of the ectoparasitoid *Nasonia vitripennis* (Walker) (Hymenoptera, Pteromalidae). *Journal of Invertebrate Pathology,* **66,** 104-110.

Roitberg, B.D., Mangel, M., Lalonde, R.G., Roitberg, C.A., van Alphen, J.J.M. & Vet, L. (1992) Seasonal dynamic shifts in patch exploitation by parasitic wasps. *Behavioral Ecology,* **3,** 156-165.

Roitberg, B.D., Sircom, J., Roitberg, C.A., van Alphen, J.J.M. & Mangel, M. (1993) Life expectancy and reproduction. *Nature,* **364,** 108-108.

Roitberg, B.D., Zimmermann, K. & Hoffmeister, T.S. (2010) Dynamic response to danger in a parasitoid wasp. *Behavioral Ecology and Sociobiology,* **64,** 627-637.

Rosenheim, J.A. (1996) An evolutionary argument for egg limitation. *Evolution,* **50,** 2089-2094.

Rosenheim, J.A. (1999) The relative contributions of time and eggs to the cost of reproduction. *Evolution,* **53,** 376-385.

Rosenheim, J.A. (2011) Stochasticity in reproductive opportunity and the evolution of egg limitation in insects. *Evolution,* **65,** 2300-2312.

Rosenheim, J.A., Heimpel, G.E. & Mangel, M. (2000) Egg maturation, egg resorption and the costliness of transient egg limitation in insects. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **267,** 1565-1573.

Rosenheim, J.A., Jepsen, S.J., Matthews, C.E., Smith, D.S. & Rosenheim, M.R. (2008) Time limitation, egg limitation, the cost of oviposition, and lifetime reproduction by an insect in nature. *American Naturalist,* **172,** 486-496.

Rosenheim, J.A. & Rosen, D. (1991) Foraging and oviposition decisions in the parasitoid *Aphytis lingnanensis* - distinguishing the influences of egg load and experience. *Journal of Animal Ecology,* **60,** 873-893.

Schmidt, J.M. & Smith, J.J.B. (1985) Host volume measurement by the parasitoid wasp *Trichogramma minutum*: the roles of curvature and surfacearea. *Entomologia Experimentalis Et Applicata,* **39,** 213-221.

Schmidt, O., Theopold, U. & Strand, M. (2001) Innate immunity and its evasion and suppression by hymenopteran endoparasitoids. *BioEssays,* **23,** 344-351.

Schurmann, D., Collatz, J., Hagenbucher, S., Ruther, J. & Steidle, J.L.M. (2009) Olfactory host finding, intermediate memory and its potential ecological adaptation in *Nasonia vitripennis*. *Naturwissenschaften,* **96,** 383-391.

Schurmann, D., Sommer, C., Schinko, A.P.B., Greschista, M., Smid, H. & Steidle, J.L.M. (2012) Demonstration of long-term memory in the parasitic wasp *Nasonia vitripennis*. *Entomologia Experimentalis Et Applicata,* **143,** 199-206.

Segoli, M. & Rosenheim, J.A. (2013) Limits to the reproductive success of two insect parasitoid species in the field. *Ecology,* **94,** 2498-2504.

Sevenster, J.G., Ellers, J. & Driessen, G. (1998) An evolutionary argument for time limitation. *Evolution,* **52,** 1241-1244.

Sherry, D.F. & Mitchell, J.B. (2007) 3 Neuroethology of foraging. *Foraging: Behavior and Ecology* (eds D.W. Stephens, J.S. Brown & R.C. Ydenberg). University of Chicago Press, Chicago.

Shettleworth, S.J. (2010) *Cognition, evolution, and behavior,* 2 edn. Oxford University Press, New York.

Shuker, D.M., Pen, I., Duncan, A.B., Reece, S.E. & West, S.A. (2005) Sex ratios under asymmetrical local mate competition: theory and a test with parasitoid wasps. *American Naturalist,* **166,** 301-316.

Shuker, D.M., Pen, I. & West, S.A. (2006) Sex ratios under asymmetrical local mate competition in the parasitoid wasp *Nasonia vitripennis*. *Behavioral Ecology,* **17,** 345-352.

Shuker, D.M., Reece, S.E., Lee, A., Graham, A., Duncan, A.B. & West, S.A. (2007) Information use in space and time: sex allocation behaviour in the parasitoid wasp *Nasonia vitripennis*. *Animal Behaviour,* **73,** 971-977.

Shuker, D.M. & West, S.A. (2004) Information constraints and the precision of adaptation: Sex ratio manipulation in wasps. *Proceedings of the National Academy of Sciences of the United States of America,* **101,** 10363-10367.

Skinner, S.W. (1985) Clutch size as an optimal foraging problem for insects. *Behavioral Ecology and Sociobiology,* **17,** 231-238.

Smid, H.M., Wang, G.H., Bukovinszky, T., Steidle, J.L.M., Bleeker, M.A.K., van Loon, J.J.A. & Vet, L.E.M. (2007) Species-specific acquisition and consolidation of long-term memory in parasitic wasps. *Proceedings of the Royal Society B-Biological Sciences,* **274,** 1539-1546.

Smith, G.J.C. (1969) Host selection and oviposition behavior of *Nasonia vitripennis* (Hymenoptera: Pteromalidae) on two host species. *The Canadian Entomologist,* **101,** 533-538.

Snell-Rood, E.C., Davidowitz, G. & Papaj, D.R. (2011) Reproductive tradeoffs of learning in a butterfly. *Behavioral Ecology,* **22,** 291-302.

Steidle, J.L.M. (1998) Learning pays off: influence of experience on host finding and parasitism in *Lariophagus distinguendus*. *Ecological Entomology,* **23,** 451-456.

Stephens, D., W. (1991) Change, regularity, and value in the evolution of animal learning. *Behavioral Ecology,* **2,** 77-89.

Stephens, P.A., Sutherland, W.J. & Freckleton, R.P. (1999) What is the Allee effect? *Oikos,* **87,** 185-190.

Sykes, E.M., Innocent, T.M., Pen, I., Shuker, D.M. & West, S.A. (2007) Asymmetric larval competition in the parasitoid wasp *Nasonia vitripennis*: a role in sex allocation? *Behavioral Ecology and Sociobiology,* **61,** 1751-1758.

Takagi, M. (1985) The reproductive strategy of the gregarious parasitoid, Pteromalus puparum (Hymenoptera, Pteromalidae). 1. Optimal number of eggs in a single host. *Oecologia,* **68,** 1-6.

Takasu, K. & Lewis, W.J. (2003) Learning of host searching cues by the larval parasitoid *Microplitis croceipes*. *Entomologia Experimentalis Et Applicata,* **108,** 77-86.

Tamura, T., Chiang, A.S., Ito, N., Liu, H.P., Horiuchi, J., Tully, T. & Saitoe, M. (2003) Aging specifically impairs amnesiac-dependent memory in *Drosophila*. *Neuron,* **40,** 1003-1011.

Tentelier, C., Desouhant, E. & Fauvergue, X. (2006) Habitat assessment by parasitoids: mechanisms for patch use behavior. *Behavioral Ecology,* **17,** 515-521.

Tentelier, C., Lacroix, M.N. & Fauvergue, X. (2009) Inflexible wasps: the aphid parasitoid *Lysiphlebus testaceipes* does not track multiple changes in habitat profitability. *Animal Behaviour,* **77,** 95-100.

Thiel, A. (2011) How to measure patch encounter rate: decision-making mechanisms in the parasitic wasp *Asobara tabida*. *Animal Cognition,* **14,** 73-82.

Thiel, A., Driessen, G. & Hoffmeister, T.S. (2006) Different habitats, different habits? Response to foraging information in the parasitic wasp *Venturia canescens*. *Behavioral Ecology and Sociobiology,* **59,** 614-623.

Thiel, A. & Hoffmeister, T.S. (2004) Knowing your habitat: linking patch-encounter rate and patch exploitation in parasitoids. *Behavioral Ecology,* **15,** 419-425.

Thiel, A. & Hoffmeister, T.S. (2006) Selective information use in parasitoid wasps. *Animal Biology,* **56,** 233-245.

Thiel, A., Schlake, S. & Kosior, D. (2013) Omnia tempus habent: habitat-specific differences in olfactory learning and decision making in parasitic wasps. *Animal Cognition,* **16,** 223-232.

Tully, T., Preat, T., Boynton, S.C. & Delvecchio, M. (1994) Genetic dissection of consolidated memory in *Drosophila*. *Cell,* **79,** 35-47.

Urbanek, S. & Horner, J. (2013) Cairo: R graphics device using cairo graphics library for creating high-quality bitmap (PNG, JPEG, TIFF), vector (PDF, SVG, PostScript) and display (X11 and Win32) output. *R package version 1.5-5*.

van Alpen, J.J.M., Bernstein, C. & Driessen, G. (2003) Information acquisition and time allocation in insect parasitoids. *Trends in Ecology & Evolution,* **18,** 81-87.

van de Zande, L., Ferber, S., de Haan, A., Beukeboom, L.W., van Heerwaarden, J. & Pannebakker, B.A. (2014) Development of a *Nasonia vitripennis* outbred laboratory population for genetic analysis. *Molecular Ecology Resources,* **14,** 578-587.

van den Berg, M., Duivenvoorde, L., Wang, G.H., Tribuhl, S., Bukovinszky, T., Vet, L.E.M., Dicke, M. & Smid, H.M. (2011) Natural variation in learning and memory dynamics studied by artificial selection on learning rate in parasitic wasps. *Animal Behaviour,* **81,** 325-333.

van den Berg, M., Verbaarschot, P., Hontelez, S., Vet, L.E.M., Dicke, M. & Smid, H.M. (2010) CREB expression in the brains of two closely related parasitic wasp species that differ in long-term memory formation. *Insect Molecular Biology,* **19,** 367-379.

van Randen, E.J. & Roitberg, B.D. (1996) The effect of egg load on superparasitism by the snowberry fly. *Entomologia Experimentalis Et Applicata,* **79,** 241-245.

Vet, L.E.M., Datema, A., Janssen, A. & Snellen, H. (1994) Clutch size in a larval-pupal endoparasitoid: Consequences for fitness. *Journal of Animal Ecology,* **63,** 807-815.

Vet, L.E.M., Wäckers, F.L. & Dicke, M. (1991) How to hunt for hiding hosts - the reliability-detectability problem in foraging parasitoids. *Netherlands Journal of Zoology,* **41,** 202-213.

Visser, M.E. (1994) The importance of being large - the relationship between size and fitness in females of the parasitoid Aphaereta minuta (Hymenoptera, Braconidae). *Journal of Animal Ecology,* **63,** 963-978.

Visser, M.E., van Alphen, J.J.M. & Nell, H.W. (1992) Adaptive superparasitism and patch time allocation in solitary parasitoids - the influence of prepatch experience. *Behavioral Ecology and Sociobiology,* **31,** 163-171.

Voss, S.C., Spafford, H. & Dadour, I.R. (2009) Hymenopteran parasitoids of forensic importance: host associations, seasonality, and prevalence of parasitoids of carrion flies in Western Australia. *Journal of Medical Entomology,* **46,** 1210-1219.

Voss, S.C., Spafford, H. & Dadour, I.R. (2010) Temperature-dependant development of *Nasonia vitripennis* on five forensically important carrion fly species. *Entomologia Experimentalis Et Applicata,* **135,** 37-47.

Wajnberg, E. (2006) Time allocation strategies in insect parasitoids: from ultimate predictions to proximate behavioral mechanisms. *Behavioral Ecology and Sociobiology,* **60,** 589-611.

Wajnberg, E., Gonsard, P.A., Tabone, E., Curty, C., Lezcano, N. & Colazza, S. (2003) A comparative analysis of patch-leaving decision rules in a parasitoid family. *Journal of Animal Ecology,* **72,** 618-626.

Warnes, G.R. (2012) gplots: various R programming tools for plotting data.

Warnes, G.R., Bolker, B., Bonebakker, L., Gentleman, R., Liaw, W.H.A., Lumley, T., Maechler, M., Magnusson, A., Moeller, S., Schwartz, M. & Venables, B. (2014) gplots: Various R programming tools for plotting data. *R package version 2.14.0*.

Werren, J.H. (1980) Sex-ratio adaptations to local mate competition in a parasitic wasp. *Science,* **208,** 1157-1159.

Werren, J.H. (1984) Brood size and sex ratio regulation in the parasitic wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). *Netherlands Journal of Zoology,* **34,** 123-143.

Werren, J.H. & Loehlin, D.W. (2009a) The parasitoid wasp *Nasonia*: an emerging model system with haploid male genetics. *Cold Spring Harbor Protocols,* **2009,** pdb.emo134.

Werren, J.H. & Loehlin, D.W. (2009b) Strain maintenance of *Nasonia vitripennis* (parasitoid wasp). *Cold Spring Harbor Protocols,* **2009,** pdb.prot5307.

Werren, J.H. & Loehlin, D.W. (2009c) Virgin collection and haplodiploid crossing methods in *Nasonia* (parasitoid wasp). *Cold Spring Harbor Protocols,* **2009,** pdb.prot5310.

Werren, J.H., Richards, S., Desjardins, C.A., Niehuis, O., Gadau, J., et al. (2010) Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species. *Science,* **327,** 343-348.

West, S.A., Flanagan, K.E. & Godfray, H.C.J. (1996) The relationship between parasitoid size and fitness in the field, a study of *Achrysocharoides zwoelferi* (Hymenoptera: Eulophidae). *Journal of Animal Ecology,* **65,** 631-639.

West, S.A., Flanagan, K.E. & Godfray, H.C.J. (2001) Variable host quality, life-history invariants, and the reproductive strategy of a parasitoid wasp that produces single sex clutches. *Behavioral Ecology,* **12,** 577-583.

Whiting, A.R. (1967) Biology of parasitic wasp *Mormoniella vitripennis* [=*Nasonia brevicornis*] (Walker). *Quarterly Review of Biology,* **42,** 333-406.

Withers, T.M., Madie, C. & Harris, M.O. (1997) The influence of clutch size on survival and reproductive potential of Hessian fly. *Entomologia Experimentalis Et Applicata,* **83,** 205-212.

Wylie, H.G. (1963) Some effects of host age on parasitism by *Nasonia vitripennis* (Walk.) (Hymenoptera: Pteromalidae). *The Canadian Entomologist,* **95,** 881-886.

Wylie, H.G. (1966) Survival and reproduction of *Nasonia vitripennis* (Walk.) at different host population densities. *Canadian Entomologist,* **98,** 275-281.

Wylie, H.G. (1967) Some effects of host size on *Nasonia vitripennis* and *Muscidifurax raptor* (Hymenoptera - Pteromalidae). *Canadian Entomologist,* **99,** 742-748.

Xia, S.Z., Feng, C.H. & Guo, A.K. (1998) Multiple-phase model of memory consolidation confirmed by behavioral and pharmacological analyses of operant conditioning in *Drosophila*. *Pharmacology Biochemistry and Behavior,* **60,** 809-816.

Xia, S.Z., Feng, C.H. & Guo, A.K. (1999) Temporary amnesia induced by cold anesthesia and hypoxia in Drosophila. *Physiology & Behavior,* **65,** 617-623.

Xu, L.L., Zhou, C.M., Xiao, Y., Zhang, P.F., Tang, Y. & Xu, Y.J. (2012) Insect oviposition plasticity in response to host availability: the case of the tephritid fruit fly *Bactrocera dorsalis*. *Ecological Entomology,* **37,** 446-452.

Ydenberg, R.C., Brown, J.S. & Stephens, D.W. (2007) 1 Foraging: an overview. *Foraging: Behavior and Ecology* (eds D.W. Stephens, J.S. Brown & R.C. Ydenberg). University of Chicago Press, Chicago.

Ye, J.L., Zhao, H.W., Wang, H.J., Bian, J.M. & Zheng, R.Q. (2010) A defensin antimicrobial peptide from the venoms of *Nasonia vitripennis*. *Toxicon,* **56,** 101-106.

Zaviezo, T. & Mills, N. (2000) Factors influencing the evolution of clutch size in a gregarious insect parasitoid. *Journal of Animal Ecology,* **69,** 1047-1057.

Zeileis, A. & Hothorn, T. (2002) Diagnostic checking in regression relationships. *R News,* **2,** 7-10.

Zeileis, A., Kleiber, C. & Jackman, S. (2008) Regression models for count data in R. *Journal of Statistical Software,* **27,** 1-25.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. & Smith, G.M. (2009) *Mixed effects models and extensions in ecology with R*. Springer, New York, NY.

8.2 Acknowledgements

First of all I would like to thank **Thomas Hoffmeister** for supervising my PhD thesis, I am honestly grateful for the opportunity he has given me. I also want to thank Thomas for encouragement, when desperately needed, for gently pushing me to go the necessary steps to become a scientist and for teaching me a great deal about parasitoids, statistics, behavioral ecology and probably many other things I am not even aware of.

Many thanks go to **Andra Thiel** for writing the proposal for the grant that made this project possible and for usually giving immediate feedback on my writing attempts. Our often opposing opinions helped me to strengthen my arguments and discriminate between my good and my less brilliant thoughts.

My thanks also go to the whole **Population & Evolutionary Ecology Lab** for helpful discussions throughout the whole project. I especially thank **Erika Lorenz** and **Laura Rogge**. Erika helped me to find my way around the lab, when I started my project. Laura was a great help during experiments and good company during coffee breaks and tram rides home.

I would like to thank **Professor Stick** for sharing his facilities during my experiments with injections of memory inhibitors. I am especially grateful to **Annette Peter** who patiently helped me with my first attempts using the microinjector.

Many thanks go to **Sven Brunkhorst**, **Eike Gerhard**, **Katrin Panzer** and **Melanie Kieß** for doing their Bachelor/Diplom theses on *Nasonia* learning behavior, contributing valuable data to the project and temporarily sharing the fun of working with *Nasonia*. I would also like to thank two student helpers, **Lena Crummenerl** and **Anne Paa**, for their careful work.

I would like to thank the members of the ***Nasonia* community**, especially **Hannes Steidle** and **Alexander Schinko** (University of Hohenheim) for sharing recent insights, providing helpful discussions and Alexander for teaching me his microinjection technique, **Hans Smid** and **Katja Hoedjes** (University of Wageningen) for helpful discussions on *Nasonia* learning and memory formation and **Bart Pannebakker** (University of Wageningen) for sharing the *Nasonia* strains with us.

I am grateful to **Katharina Merkel** and **Sven Hauk** for being perfect office mates, keeping my caffeine level high and taking care of our chocolate supplies. Special thanks to Katharina for listening to all my ideas, doubts and complaints, for seeking and giving feedback on all the aspects of our work and for sharing the joy of doing statistics and working with R.

Thanks to **Claudia Hogekamp**, **Tim Grotjohann** and **Ingo Appelhagen** who have made living in Bielefeld great fun during our studies and who have endured all my doubts during the years after.

I am grateful to **my parents** who always supported me in my plans and decisions.

Many thanks to my sister, **Imke**, not only for being the best sister one could wish for but also for repeatedly sharing her one room apartment with me during my first months in Bremen.

This project was funded by the Central Research Development Fund, University of Bremen, grant 02/125/10 to Andra Thiel.

8.3 Contributions of authors

1 Summaries

Mareike Koppik

This chapter was written by myself.

2 General introduction

Mareike Koppik

This chapter was written by myself, T.S. Hoffmeister provided helpful comments.

3 Adaptive decision making or differential mortality: what causes offspring emergence in a gregarious parasitoid?

Mareike Koppik, Andra Thiel & Thomas S. Hoffmeister

I designed the experiment (with discussions with AT and TSH), collected the data, did the statistical analysis (with discussions with AT and TSH) and prepared the manuscript with comments and editing by AT and TSH, two anonymous reviewers provided helpful comments on the manuscript.

4 Causes of intraspecific variability in associative learning in the parasitic wasp *Nasonia vitripennis*

Mareike Koppik, Thomas S. Hoffmeister, Sven Brunkhorst, Melanie Kieß & Andra Thiel

I designed the experiments with TSH (with discussions with SB, MKi and AT), collected the data with SB and MKi, did the statistical analysis (with discussions with TSH and AT) and prepared the manuscript with comments and editing by TSH and AT, two anonymous reviewers provided helpful comments on the manuscript.

5 Clutch size variation in the parasitic wasp *Nasonia vitripennis* in response to physiological state and the information content in host experience

Mareike Koppik, Andra Thiel & Thomas S. Hoffmeister

I designed the experiment (with discussions with AT and TSH), collected the data, did the statistical analysis (with discussions with AT and TSH) and prepared the manuscript with comments and editing by AT and TSH.

6 Wait and see: time allocation under egg limitation in the synovigenic parasitoid ***Nasonia vitripennis***

Mareike Koppik, Thomas S. Hoffmeister & Andra Thiel

I designed the experiment (with discussions with TSH and AT), collected the data and did most of the statistical analysis (with discussions with TSH and AT), initial analyses with GLMMs was done by TSH. I prepared the manuscript with comments and editing by TSH and AT.

7 General discussion and conclusions

Mareike Koppik

This chapter was written by myself, T.S. Hoffmeister provided helpful comments.

8.4 Erklärung

Name: Mareike Koppik Ort, Datum: ____________________

Anschrift: Moselstraße 25, 28199 Bremen

ERKLÄRUNG

Hiermit erkläre ich, dass ich die Doktorarbeit mit dem Titel:

„Information use and memory formation during foraging in the parasitoid *Nasonia vitripennis*“

selbständig verfasst und geschrieben habe und außer den angegebenen Quellen keine weiteren Hilfsmittel verwendet habe.

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

____

(Unterschrift)