On handling costs and host choice in aphid-parasitoids:

from individual behaviour to evolutionary patterns

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“I venture another, and perhaps equally reckless, generalization - nothing makes sense in biology except in the light of evolution, *sub specie evolutionis.*"

(Dobzhansky 1964)

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ABSTRACT

Foraging animals incur handling costs when capturing, subduing, or killing their resources. Handling costs are hypothesized to influence the dietary choices of animals and influence the structure of ecological communities. It is not clear, however, whether trophic interactions found in communities correspond to individual decisions. This thesis investigated the determinants of handling costs and their consequences for host choice by aphid parasitoids (Hymenoptera, Braconidae, Aphidiinae) at the level of individuals and communities.

Laboratory experiments using the parasitoid *Aphidius colemani* showed that the cost of handling a host aphid (*Myzus persicae*) is inversely related to the parasitoid:host body size ratio. Further experiments showed that developmental temperature influences the handling time of parasitoids by affecting parasitoid body size. The defences of aphids are expected to impose a handling cost to parasitoids. The cornicle secretions of the aphid *Sitobion avenae*, however, did not increase the handling time of the parasitoid *A. rhopalosiphi* in laboratory experiments. This is likely because cornicle secretions have an altruistic function rather than self-preservation. Hence, consumer:resource body size ratio seems the principal determinant of handling time in aphid-parasitoid interactions. When given a choice, female parasitoids preferred hosts that maximized their foraging rate (value/handling time) as predicted by optimal foraging.

At the level of communities, the relationship between handling time and body size ratio is expected to result in a positive association between the body size of optimally foraging consumers and of their resources. Comparative studies of aphid-parasitoid revealed no relationship between handling time and body size ratio, but showed a clear positive correlation between consumer and resource body sizes. Further phylogenetic analyses revealed that the correlation between aphid and parasitoid body sizes can be attributed entirely to their evolutionary history (phylogeny).

This thesis showed that body sizes of aphids and parasitoids influence handling costs and host choices, but that this result does not scale up to ecological communities. Rather, the host choice of parasitoids for different species of aphids is explained by phylogeny. I discuss the potential implication of these results for scaling behaviour and for applied ecology.

RÉSUMÉ

L’exploitation des ressources impose un coût de manipulation pour capturer, poursuivre, ou tuer une ressource. Les coûts de manipulation devraient influencer les choix des animaux et conséquemment, la structure des communautés. Le comportement d’exploitation au niveau individuel pourrait ne pas être valide pour les communautés où plusieurs espèces interagissent. L’objectif de cette thèse est de déterminer les facteurs influençant le coût de manipulation chez les parasitoïdes de puceron (Hymenoptera, Braconidae, Aphidiinae) et les conséquences pour le choix d’hôte au niveau individuel et des communautés.

Des expériences en laboratoire sur le parasitoïde *Aphidius colemani* ont démontré que le coût de manipulation d’un hôte (*Myzus persicae*), diminue lorsque le ratio de taille parasitoïde:puceron augmente. De plus, la température de développement des parasitoïdes influence leur temps de manipulation en modifiant leur taille corporelle. Les défenses des hôtes devraient influencer le temps de manipulation des parasitoïdes, mais des expériences en laboratoire ont démontré que l’utilisation de sécrétions corniculaires par le puceron *Sitobion avenae* n’affecte pas le temps de manipulation du parasitoïde *Aphidius rhopalosiphi*. Ce résultat serait lié à la fonction altruiste des sécrétions corniculaires. Il semblerait donc que le ratio de taille consommateur:ressource soit le principal facteur influençant le coût de manipulation des parasitoïdes de puceron.

Au niveau des communautés, cette relation devrait donner lieu à une corrélation positive entre la taille corporelle des parasitoïdes et de leurs hôtes. Des analyses comparatives ont démontré que le coût de manipulation n’est pas lié au ratio de taille consommateur:ressource à l’échelle de la communauté, mais que la taille corporelle des parasitoïdes et de leurs hôtes sont néanmoins positivement corrélées. Par contre, cette corrélation peut être attribuée complètement au passée évolutif (phylogénie) des animaux plutôt qu’à leur choix d’hôte.

Cette thèse a démontré que la taille corporelle des pucerons et des parasitoïdes influence le coût de manipulation et le choix des hôtes des parasitoïdes, mais que ce résultat ne s'applique pas à l'échelle des communautés. Le choix d'hôte lorsque différentes espèces de puceron sont présentes serait plutôt expliqué par la phylogénie. Je discute des conséquences de ces résultats pour l'étude du comportement à plusieurs échelles.

HEARTFELT ACKNOWLEDGEMENTS

I was once told that academia was like a second family (Luc-Alain Giraldeau). The meaning of this becomes all too clear when I think of all the bonds that have formed; every person has been supportive and an essential part of this journey. The following words can only hint at my sincere and heartfelt thanks to my extended family.

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To the many grad students and technicians (some Ph.D.'s and many friends now) who made the office, lab, happy hours, and scientific meetings so enjoyable: If it is conceivable, I hope I have given you at least as much in return! Special thanks to Annabelle Firlej for her insights during many discussions; Véronique Martel for sharing the drive to St-Jean; David Damiens, Sara Ruschoni, Josée Doyon, Léna Durocher, Marie-Pier Lepage, Marie-Jude Merisier, and of course Maryse Barrette, collaborator and best conference-buddy! Many experiments were only possible thanks to Danielle Thibodault, Josianne Vaillancourt, Julie Frenette, Michel Fortin, and Hervé Colinet. Without your technical expertise, I surely would have messed up many (more) experiments. Likewise, the comparative analyses would not have been possible without the help of Robert Foottit, Eric Maw, and Henry Goulet with the Canadian National Collection of Insects, and

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Finally, I am in debt to McGill University, NSERC, the Biocontrol Network of Canada, Centre Sève, the Wallonie-Bruxelles International grant, the Ministère de l’Agriculture (France), the Ministère de l’Éducation, de la Recherche et de la Technologie (France) for their financial contribution.

CONTRIBUTIONS TO KNOWLEDGE

This research resulted in the following original contributions to knowledge:

1. Handling time has been hypothesized to be inversely related to the consumer:resource body size ratio (Griffiths 1980b), but explicit tests of the relationship is not common, especially in parasitoid wasps. Chapter 3 provides this test in the parasitoid *Aphidius colemani* (Hymenoptera, Braconidae, Aphidiinae). In addition, it extends the relevance of this result to developmental temperature by showing that developmental temperature influences handling time through body size-mediated effects. These results should be of wide relevance for the foraging behaviour of most ectotherms.
2. The defence behaviour of potential prey or hosts are expected to increase their probability of survival or at least reduce their profitability below the acceptance threshold of a consumer. Chapter 4 shows how the use of cornicle secretions by the aphid *Sitobion avenae* does not provide them any benefit in terms of increased survival or reduced profitability for the parasitoid *Aphidius rhopalosiphi*. Results shows that this seemingly unadaptive trait can evolve because it is altruistic. While few defence behaviours are expected to be altruistic outside eusocial animals, the use of cornicle secretions is common in aphids.
3. The optimal diet model predicts the prey (or host) choice of animals based on the profitability of available prey (benefit/cost), but the usefulness of the model has often been limited by a poor evaluation of costs (handling time) in particular (Sih and Christensen 2001). Chapter 5 provides a detailed quantitative evaluation of benefits and costs of different sized aphids (*Myzus persicae*) for the parasitoid *Aphidius colemani*, and provides support for the optimal diet model.

4. Theoretical studies of ecological communities are increasingly including explicitly foraging behaviour in their models. One of the key assumptions in the models is that handling time is inversely related to consumer:resource body size ratio, and assuming a different relationship changes the predictions of the models considerably (Petchey et al. 2008). Very few studies have tested this relationship for interactions between multiple species, especially using the phylogenetic methods that are required for such interactions. Chapter 6 uses a phylogenetic comparative study to show that the relationship between handling time and body size ratio does not hold for interactions among multiple species of aphids and parasitoids.
5. The optimal diet model predicts a positive correlation between the body size of consumers and of their resources, which is often observed in ecological communities. However, this relationship has never been tested using phylogenetic methods, often because communities are assumed to form through ecological processes. Chapter 7 analyzes the relationship between the body sizes of associated parasitoid and aphid species from the large data set of a regional community. The results show the typical positive body size relationship, but also that the relationship can be attributed completely to phylogeny.

CONTRIBUTIONS OF AUTHORS

All chapters are co-authored papers submitted or to be submitted, and many were the result of enjoyable collaborations. Ideas for all the papers were discussed among all co-authors and the rest of the contributions for each chapter are detailed in the following paragraphs. All co-authors have revised their respective manuscripts.

Chapter 3 "Temperature affects handling time through body size-mediated effects": The ideas for the influence of temperature were first discussed between Dr. Hance and I. Experiments were conducted by M. Barrette and I. I performed all the analyses and wrote the paper.

Chapter 4 "Altruistic defence behaviours in aphids": The experiment was performed by Dr. Outreman, and I had the idea to test for the altruistic nature of cornicle secretions, performed all the analyses and wrote the paper.

Chapter 5 "Testing competing measures of profitability for mobile resources": Experiments and analyses were shared equally by M. Barrette and I except for the preference tests, which M. Barrette did. M. Barrette was the lead author on this paper.

Chapter 6 and 7 "Body size of host, but not parasitoids determines handling time in aphid-parasitoid interactions" and "Phylogeny explains body size relationships in aphid-parasitoid associations": I took measurements from museum specimens, gathered the data from the literature, performed the analyses, and wrote the papers.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

The decision to accept or to reject a resource item (diet choice) is a fundamental part of the behaviour of most animals. Be it for a potential meal or host (a meal for the offspring of parasites or parasitoids), diet choice is expected to have consequences for the fitness of animals and thus, to have been under natural selection. Optimal foraging theory hypothesizes that diet choice maximizes an animal's fitness (Charnov 1976a; Emlen 1966; MacArthur and Pianka 1966; Stephens et al. 2007; Ydenberg et al. 2007), and increasingly, that these decisions influence higher level of organisation such as community structure (Brose 2010; Petchey et al. 2008). Many questions remain unresolved about the dietary choices of animals and how they influence characteristics of communities.

Classical optimality models of diet choice predict that foragers should only accept resource items (prey or hosts) whose profitability (benefit / handling time) is greater than the expected rate of energy intake achieved in the environment by including only prey types of higher profitability (Charnov 1976a; Emlen 1966). Many studies provide support for the optimal diet model in animals (Stephens and Krebs 1986). Nevertheless, tests of the optimal diet model often fail when foragers exploit mobile resources, presumably because most tests neglect handling costs such as the time required to capture or subdue a resource that can defend or flee (Christensen 1996; Sih and Christensen 2001). The profitability of mobile resources can therefore be overestimated so that their inclusion in a forager's diet is wrongly predicted. Tests of the optimal diet model therefore require a particularly good understanding of handling costs in the model system used. Handling time is expected to decrease with the relative body size of the forager or the ratio of consumer:resource body size (Griffiths 1980b), and does so in some systems (Gill and Hart 1994; Griffiths 1980a). It follows that factors influencing the body size of animals may also affect a forager's efficacy at handling resource items. In the case of ectothermic foragers for instance, body size at maturity is often inversely correlated to the temperature experienced during development as described by the temperature-size rule (Atkinson 1994; Atkinson and Sibly 1997; Walters and Hassall 2006).

Individual dietary choices of animals have been hypothesized to "scale up" (Stephens 1990; Williams et al. 2007) or shape higher levels of organisation such as community dynamics. Few studies have modelled explicitly the effect of individual dietary choices on the structure of communities until recently (Beckerman et al. 2006; Brose 2010; Petchey et al. 2008). Foraging models of ecological communities suggest that handling time plays a central role in determining who eats whom in a food web (Petchey et al. 2008). Consequently, the maximum size of resource used by a consumer is predicted to increase with its own body size. The positive relationship between the body sizes of consumer and resource species found in many food webs is consistent with this hypothesis (Brose et al. 2006), but does not constitute strong evidence. Handling time is inversely related to consumer:resource body size ratio in many studies that focus on single trophic interactions (one species of consumer and one species of resource); this may be different in multi-species trophic interactions. Species-specific adaptations can blur or annul the relationship between handling time and body size. Many animals specialize on certain types of resources and their behavioural and morphological adaptations can make body size less relevant to handling time. Moreover, the relationship between the size of consumers and their resources may be confounded by evolutionary history (phylogeny) especially since body size is often conserved among closely related species of animals (Ashton 2004; Blomberg et al. 2003; Freckleton et al. 2002). Dietary choice may be a phylogenetically conserved trait (Brandl et al. 1994; Freckleton et al. 2002) such that closely related species share similar body sizes and utilize resource species of similar sizes simply due to their shared phylogeny. Alternatively, competition may have favoured the evolution of divergent traits so that closely related species may have very different diets. Very few studies of trophic interactions have taken phylogeny into consideration and for those that have, only the phylogeny of consumers, not that of resources, has been considered (Brandl et al. 1994; Rezende et al. 2009). Studying the pattern of body size evolution could help distinguish the contributions of phylogeny and current ecological forces in structuring a community. If the association between consumer and resource body size was only due to current ecological processes (e.g. dietary choices), then consumer body size may have no phylogenetic signal or be phylogenetically conserved. If competition influenced the evolution of body size of consumer and resource species, a pattern of disruptive selection can be expected (Futuyma and Moreno 1988).

Models of optimal diet models assume that success in foraging translates to fitness benefits, so that parasitoids are especially well-suited test organisms (Godfray and Shimada 1999). Most female parasitoids lay their eggs in or on hosts, which serve as food for their offspring so there is a close correlation between foraging success and fitness (Godfray 1994; Reuter 1913). In the field, aphid parasitoids use chemical cues of the hosts or plants on which the latter feed (Vinson 1976). Consequently, host choice decisions often result in a choice of host species. While hosts within a patch can vary in body size, host species determine the average and range of host sizes available in the patch.

1.2 Objectives

This thesis aims to understand the economics of host choices in aphid parasitoids (Hymenoptera, Braconidae, Aphidiinae) and the implications of these choices for communities (Figure 1.2.1). Two broad questions are addressed here: 1) What factors influence the handling cost of parasitism? and 2) Do parasitoids choose hosts optimally?

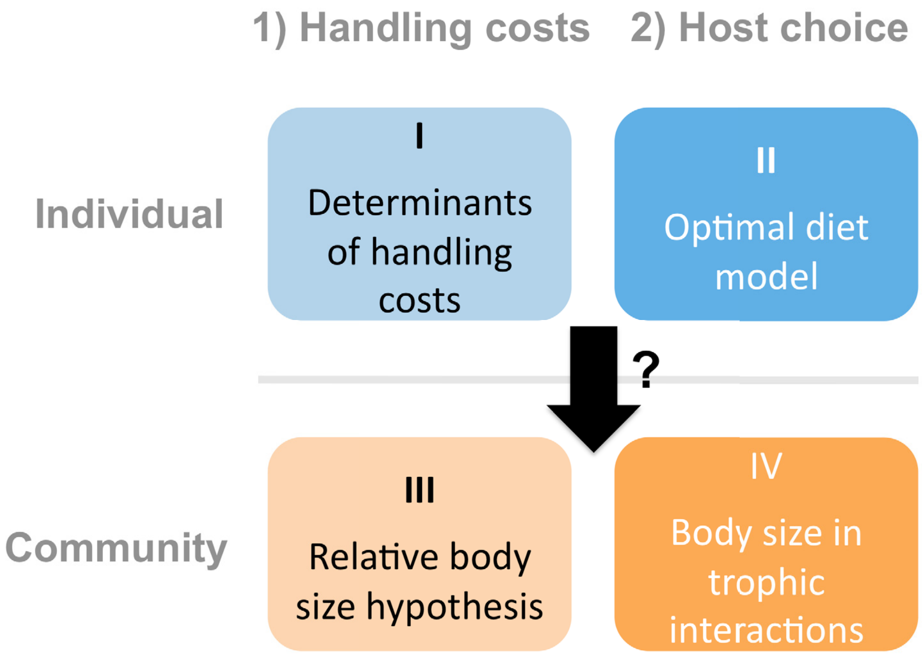

Figure 1.2.1 Diagram of the general questions addressed in this thesis. Questions about 1) determinants of handling costs and 2) host choice are addressed at the level of individuals (blue) and communities (orange). Collectively, they aim to explore the potential implications of individual foraging behaviour for higher levels of organisation, the community (black arrow).

This thesis combines laboratory experiments and phylogenetic comparative studies to explore these questions at the level of individuals and communities, respectively. Specifically, the objectives are to:

I. Identify determinants of handling time using laboratory experiments (Chapters 3 and 4)
II. Determine the role of handling cost in individual host choice using laboratory experiments (Chapter 5)
III. Determine the importance of body size for handling cost in multi-species interactions using a phylogenetic comparative study (Chapter 6)

IV. Determine the potential implications of optimal dietary choices for patterns of body size relationships at the level of communities using a phylogenetic comparative study (Chapter 7).

1.3 Hypotheses

This thesis tests the hypotheses that body size is a major determinant of handling costs in aphid parasitoids and that aphid parasitoids choose hosts optimally. Parasitoids should therefore prefer host sizes that maximize their rate of fitness gain. Specifically, this thesis tests the following predictions at the level of individual behaviour:

I. Handling time of female parasitoids decreases with increasing parasitoid size and increases with increasing host size.
II. Female parasitoids prefer host sizes that yield a higher fitness gain rate to those yielding a higher absolute gain.

and at the level of ecological communities:

III. Handling time decreases with increasing parasitoid species size and increases with increasing host species size.
IV. The size of associated parasitoids and hosts is positively correlated in ecological communities.

1.4 Thesis format

This thesis is manuscript-based. Each chapter has been published in, submitted to, or will be submitted to peer-reviewed scientific journals as specified in their respective preface. Chapters are the result of collaborative work and the contribution of each author is given in the “CONTRIBUTION OF AUTHORS” section.

CHAPTER 2

LITERATURE REVIEW

2.1 Foraging Theory

Foraging theory aims to understand the behaviour of animals exploiting resources, which can be food or hosts. Because obtaining food, hosts or other resources is fundamental to the survival and reproduction of animals, classical optimality models assume that foraging behaviour has been selected to maximize fitness or lifetime reproductive success (Fellowes et al. 2005; Giraldeau 2008; Stephens and Krebs 1986; Ydenberg et al. 2007). These models find the foraging strategy (decision) that maximizes a given currency of fitness, often the mean rate of food intake, for a given set of constraints (Krebs and Davies 1997). One of the central questions in foraging theory is which types of resource an animal should accept when they are encountered (Hughes 1993; Pyke et al. 1977; Stephens and Krebs 1986), and this has been modelled since the beginnings of optimal foraging theory (Emlen 1966; MacArthur and Pianka 1966).

2.1.1 Optimal diet model

The basic optimal diet models (Charnov 1976a; Emlen 1966; MacArthur and Pianka 1966) take the simple case of a solitary forager in an environment that contains different prey types. Models usually assume random and sequential encounters with prey items, and find which prey types a forager should accept (decision) to maximize its mean rate of intake (currency). Each food type i is characterized by an energetic value E_i, requires a handling time h_i, and is encountered at a rate λ_i (constraints). The optimal diet model (Charnov 1976a) predicts that prey items should only be accepted when their profitability (E_i/h_i) is greater than the expected rate of energy intake achieved in the environment by including only prey types of higher profitability. This is because accepting a prey of low profitability incurs a lost opportunity cost: the time spent handling low profitability prey could be spent foraging in the environment and achieving a higher energy intake rate. The simple prey model makes three predictions: 1) Prey types are either always or never accepted; 2) Prey types are added to the forager's diet in order of profitability; 3) The acceptance of a prey type does not depend on its own encounter rate. Tests of the prey model are generally consistent with the qualitative predictions of the model, but quantitative predictions are not often supported (Stephens and Krebs 1986). More importantly however, the framework of optimality models provides insight on the behaviour of foraging animals (Charnov 1976a) and has allowed the discovery of many important factors that can influence the dietary choices of animals (Stephens et al. 2007). This includes motivational states related to hunger, satiation (Hughes 1993; Jeschke 2007), egg limitation in parasitoids (Heimpel and Rosenheim 1998; Minkenberg et al. 1992; Rosenheim et al. 2008), or learning and experience (Vet et al. 1995).

2.1.2 Profitability of resource items

The profitability of resource items is usually defined as the ratio between the benefits obtained from a resource item and its time cost (E_i/h_i). For predators, the benefit is often assumed to be energy, which has been shown to correlate with lifetime fitness (Blanckenhorn 1991; Lemon and Barth 1992; Morse and Stephens 1996). Animals may also be balancing nutritional needs, minimizing parasite transmission (Pfennig 2000), minimizing toxicity, or minimizing the probability of incurring an energy shortfall (Stephens 1981). For female parasitoids, host selection is closely related to fitness, because host quality affects the survival and development of their offspring directly (Barrette et al. 2009; Cloutier et al. 2000; Roitberg et al. 2001), and consequently, lifetime fitness (Vos and Hemerik 2003).

Resources can take the form of food, hosts, nesting sites, etc. but independently, they all require a handling time. A forager that accepts a low profitability item can incur an opportunity cost when more profitable resources are abundant (Charnov 1976a; Emlen 1966) and foraging time is limited (Rosenheim et al. 2008). Foraging time can be limited because of unfavourable weather conditions (Weisser et al. 1997), the presence of predators or time spent on other activities. However, for predators such as snakes, digestion rate may be limiting intake rate because digestion time is several orders of magnitude greater than handling time. For female parasitoids of certain species, the availability of eggs may limit their rate of parasitism (Heimpel and Rosenheim 1998; Rosenheim et al. 2008). Costs of handling resources also include energy expenditure, but these seem relatively small when measured (Cruz-Neto et al. 2001; Rovero et al. 2000).

Although not included in the optimal diet model, handling time can also have additional costs that are not included in the optimal diet model. Flocks of birds, colonies of aphids or anemones, for instance, can offer high rates of return because of their high density. Once attacked however, defence mechanisms such as alarm signals can spread rapidly in the groups and cause birds (Caro 2005) or aphids (Wu et al. 2010) to escape or anemones to retract in their protective structures (Howe and Sheikh 1975). These aggregations can quickly dissolve or become unprofitable so that time spent handling a prey is time unavailable to exploit a rich and short-lived patch.

Time spent handling a resource item can also expose the forager to kleptoparasitism so that prey requiring long handling times may be more likely to be stolen by competitors (Ens et al. 1990). For parasitoids, time spent handling hosts in a patch may increase the alarm response of the latter and attract other parasitoids (Micha and Wyss 1996) and some but not all predators (Mondor and Roitberg 2000; Outreman et al. 2010; Verheggen et al. 2009). This is especially costly for parasitoids, because parasitized hosts remain on the patch and are vulnerable to competitors or predation (Brodeur and Rosenheim 2000) , and hyperparasitism (Sullivan 1987).

2.1.3 Determinants of handling time

Clearly, many factors influence handling time, the most intuitive and universal being body size. More specifically, the larger the consumer relative to the size of the resource, the shorter the handling time is expected to be (Griffiths 1980). This hypothesis is referred to as the "relative size hypothesis" here forth. Many studies have tested the effects of resource size or consumer size on the handling efficiency of consumers, but few have tested specifically the relative size hypothesis in predator-prey interactions (Aljetlawi et al. 2004; Christensen 1996; Gill 2003), and even fewer have done so in parasitoid-host interactions (Henry et al. 2009).

In addition to body size, certain defence mechanisms of prey or hosts such as fighting, escape responses and morphological adaptations can increase the handling time of their enemies (Jeschke and Tollrian 2000). Herons and grebes for instance, take longer to handle prey with spines to avoid injury (Forbes 1989). Even lepidopteran pupae can exhibit defensive behaviours that considerably increase the handling time of their parasitoids (Rotheray and Barbosa 1984). Most mobile prey or hosts exhibit defence behaviours (Caro 2005; Gross 1993) that impose additional handling time to their enemies. The effectiveness of physical defences such as kicking or armour likely increases with body size, but their expression often varies with ontogeny as well (Gerling et al. 1990; Walker and Hoy 2003).

2.2 Altruism

2.2.1 What is altruism

Most behavioural and morphological defences of animals have obvious fitness benefits for the individual expressing them (the actor): avoiding death. The adaptive value of certain defences is less obvious, because the actor incurs a cost, while other individuals (recipients) benefit from them. The sacrificial sting of the honeybee (*Apis mellifera* L.) exemplifies such defence; the bee stinging an animal that threatens its colony dies while affording protection to members of its colony (Breed et al. 2004 and references therein). Such behaviour is said to be altruistic and poses an evolutionary dilemma because it is, at first glance, not expected to evolve. Individuals that act selfishly could benefit from the altruistic behaviours of others while avoiding its cost. Selfish individuals are therefore expected to reproduce more successfully than altruistic individuals, so that the gene for altruism would eventually go extinct. Altruistic behaviours can evolve through kin selection (Maynard-Smith 1964; Michod 1982) when recipients of the behaviours are genetically related to the actors. Hamilton proposed that evolution maximizes inclusive fitness (Hamilton 1963; Hamilton 1964) so that altruistic behaviours that are costly to the actor (direct fitness) can evolve by increasing the fitness of genetically related individuals who are likely to share the altruistic gene (indirect fitness). The condition for the evolution of altruistic behaviours to evolve has been formalized first by Hamilton in (1963; 1964), who proposed the equation, now known as Hamilton's rule: $b > c/r$, where b is the fitness benefit obtained by the recipient, c is the cost to the actor when expressing the altruistic behaviour, and r is the genetic relatedness between the two protagonists. Altruistic behaviours can evolve through kin selection, because kin have a probability of carrying the altruistic gene and therefore, of passing it on to the next generation. More generally, altruism can evolve when recipients have a sufficiently high probability of carrying the altruistic gene regardless of kinship per se (Fletcher and Doebeli 2009; Foster et al. 2006).

2.2.2 Types of altruism

Altruism has also been defined as behaviours that benefit others, and classified according to the direct fitness consequence for the altruists (West et al. 2007b; Wilson 1990). This classification distinguishes “weak altruism” and “strong altruism”, for which the direct fitness consequences for the altruist are positive and negative, respectively. Weak altruism has also been referred to as a mutual benefit (West et al. 2007b) and is costly in terms of relative fitness: it provides higher fitness benefits to recipients than to the actor. Weak altruism can persist in a metapopulation through between-group selection when groups reform at each generation (Kerr et al. 2004; Wilson 1990). Strong altruism imposes a net direct fitness cost to the actor and is equivalent to the Hamilton’s original idea of altruism. It has been suggested that strong altruism can evolve through a new version of group selection (Wilson 1990) and multilevel selection (Fletcher and Zwick 2004), but these mechanisms have been argued to be equivalent to kin selection and remain controversial (Foster et al. 2006; Korb and Heinze 2008; Shavit and Millstein 2008). Further discussion on this topic is beyond the scope of this thesis, but can be found in a series of recent commentaries and reviews (Fletcher and Zwick 2004; Fletcher et al. 2006; Foster et al. 2006; Kerr et al. 2004; Shavit and Millstein 2008; West et al. 2007b; Wilson 2005). For the remainder of this thesis, altruism is used to refer to Hamilton’s original idea of altruism unless specifying otherwise.

2.2.3 Altruistic defences

Defence behaviours that have been proposed as examples of altruism include alarm signalling (Hollën and Radford 2009), aggressive defences

(Millor et al. 1999), and suicidal behaviours in group-living animals (Tofilski et al. 2008). The use of alarm signals has been reported in many animals, including mammals (Hollën and Radford 2009; Rose et al. 2006; Shelley and Blumstein 2005), birds (Caro et al. 2004), fish (Smith 1992), and insects (Pickett et al. 1992; Wilson 1976). When detecting the presence of a threat, an individual that emits an alarm signal can benefit its neighbours by making the latter aware of the threat. The individual emitting the signal can incurs the cost of expressing the behaviour and of making itself more conspicuous to the predator (Stern and Foster 1996). However, alarm signals can act as a selfish pursuit deterrent by communicating to the predator that the actor will be difficult to capture because of its heightened vigilance or superior condition (Caro et al. 2004; Shelley and Blumstein 2005; Wheeler 2008; Woodland et al. 1980). Altruistic defences can also include aggressive physical and chemical defences when these behaviours benefit other individuals. Aggressive defences put the actor in close proximity to the enemies and are therefore very costly. Accordingly, such behaviours are usually documented in eusocial animals. Eusocial animals are characterized by an overlap of generations, cooperative rearing of young, and non-reproducing castes (Queller and Strassmann 2003), which results in members of a group being very close kin. Aggressive defences in eusocial animals are usually expressed by specialized worker or soldier castes as found in honeybees (Breed et al. 2004), ants (Hölldobler and Wilson 1990), termites (Prestwich 1984) and eusocial aphids (Pike and Foster 2008; Stern and Foster 1996). Other striking altruistic defences include gall repair by eusocial aphids (Kutsukake et al. 2009) and nest plugging by some ants (Tofilski et al. 2008), which all result in the death of the altruist. Because these castes are usually sterile or have very little chances of reproducing, the cost in terms of their direct fitness is close to null ($c \sim 0$) so that such costly behaviours can evolve easily. Additionally, seemingly altruistic behaviours may be, at least in part, the result of selfish manipulation by the reproductive queen or other castes (Ratnieks and Wenseleers 2008).

Hamilton's rule predicts that animals should express altruistic behaviours more readily: 1) towards more closely related individuals; 2) when the benefit to kin is greater; and 3) when the cost of altruism is smaller. Most tests of kin selection have focussed on the relatedness of recipients (1) and have neglected the influence of benefits to recipients (2) and costs to the actor (3) on the expression of altruism (West et al. 2007a). In pea aphids infected by a parasitoid, the expression of a potentially altruistic behaviour (lethal escape response) was higher for second instars than for fourth instars (McAllister et al. 1990). Because infected second instar aphids have no chance of reproducing (c=0) and fourth instar aphids can give birth to a few offspring before dying (c>0), this result is consistent with the expression of altruism decreasing with an increasing cost.

2.3 Influence of temperature on ectothermic animals

Ectothermic animals have no internal means of maintaining a constant body temperature so that their metabolism and behaviour are largely dependent on the temperature of their environment (Bicego et al. 2007; Clarke and Johnston 1999; Gillooly et al. 2001; Gillooly et al. 2002). Regulation of body temperature is sometimes achieved partially through behaviours such as habitat choice, basking, or shivering, evaporative cooling (Avery et al. 1982; Heinrich 1995) and collective fanning in nests (Jones and Oldroyd 2006). Nevertheless, ectotherms remain very dependent on ambient temperature, and the resulting changes in metabolic rates have cascading effects on the animal's activity levels. For instance, the speed of locomotion and foraging efficacy (Avery et al. 1982; Avery and Mynott 1990; Byström et al. 2006) generally increase with temperature (within tolerance limits). In addition, temperature influences the growth and development of ectotherms, which has consequences on adult body size (Colinet et al. 2007; Dixon et al. 1982; Kingsolver and Huey 2008; van der Have and de Jong 1996; van Savage et al. 2004). The adult body size of ectotherms decreases with increasing developmental temperature as described by the temperature-size rule (Atkinson 1994; Atkinson and Sibly 1997; Dixon et al. 1982; Kingsolver and Huey 2008). One of several mechanisms for the relationship is thought to be a greater increase in maturation rate than in growth rate with increasing temperature, so that ectotherms reach maturity faster and at smaller body sizes in hot temperatures (Angilletta et al. 2004; Atkinson 1994; Atkinson and Sibly 1997). The temperature-size rule has been described in invertebrates (Bernal and Gonzalez 1997; Kairo and Murphy 1999b) and vertebrates (Walters and Hassall 2006). Effects of temperature on adult body size also have repercussions for traits correlated to body size such as foraging efficacy, fecundity, longevity (Bernal and Gonzalez 1997; Colinet et al. 2007), and ultimately on population dynamics (van Savage et al. 2004) or food web structure (Arim et al. 2007).

2.4 Body size in ecological communities

2.4.1 What is a community

Ecological communities are often defined as the “set of species living in the same place at the same time” (Begon et al. 1996; Fauth et al. 1996; Vellend 2010), but the term has also been restricted to species that interact together (Emerson and Gillespie 2008; Lawton 1999), or that can potentially interact (Leibold et al. 2004). This thesis follows the nomenclature proposed by Fauth (1996), which also provides terms for sets of species based on the criteria of inclusion. Basic characteristics of a community include the number, identity, and diversity of species, as well as interactions among species. Trophic interactions among species include predator-prey feeding relationships, parasitoid-host relationships, parasite-host relationships, competitive interactions, and mutualistic relationships (Begon et al. 1996).

2.4.2 Body size in communities

Studies of community ecology aim to understand patterns in characteristics of communities and their dynamics. Traditionally, two approaches have been used: 1) empirically deriving general patterns in communities and 2) modelling the processes that shape communities and give rise to those patterns (Lawton and Warren 1988; Stouffer 2010; Vellend 2010). Empirically-derived patterns include the relationships between species richness, the length of food chains, connectance, etc. (Vermaat et al. 2009), and how these vary with the spatial scale of investigation (Cornell and Lawton 1992; Martinez and Lawton 1995; Thompson and Townsend 2005). One of the most studied patterns in ecological communities is the relationship between the body sizes of interacting species. In nearly all communities of predator-prey interactions (Brose et al. 2006) the body size of consumers is positively correlated to the body size of their resource, and this is generally attributed to the central role of body size in feeding relationships. The cascade model (Cohen and Newman 1985) hypothesizes that species of animals can be ordered according to a body size-dependent hierarchy such that they only feed on all species smaller than themselves with equal probability (Cohen et al. 1993). The niche model (Williams and Martinez 2000) puts a limitation on the range of prey sizes consumed by predators (the niche), while others proposed that feeding relationships are a function of the predator:prey body size ratio (Neubert et al. 2000). While these models are somewhat successful in predicting the body-size relationships in predator-prey communities, they do not address the processes that could generate these patterns. More recently, interactions in predator-prey communities have been modelled as the result of optimal diet choices (Beckerman et al. 2006; Brose 2010; Petchey et al. 2008). The allometric diet breadth model (Petchey et al. 2008) assumes that animals maximize their rate of energy intake, and uses the relationships between body size and foraging traits (energy content, handling time, attack rates, and densities) to predict the feeding relationships in a community. The model shows that the relationship between handling time and body size plays a key role in determining feeding relationships. Furthermore, assuming that handling time is a function of predator:prey mass ratio yields food webs that best fit existing food webs and predicts a positive predator-prey body size relationship. Handling time is usually a function of consumer:resource body size ratio for given consumer and resource species, but this relationship has rarely been verified (Goss-Custard et al. 2006) In interactions among multiple species, consumers and resources can differ greatly in foraging strategies and in anti-predator adaptations, respectively.

2.4.3 Scale

Most community models assume symmetric feeding relationship among species where all species can potentially feed on each other as can be found in communities of omnivorous predators (Brose et al. 2005). Not surprisingly, these models do not predict well characteristics of communities dominated by asymmetric feeding relationships such as carnivore-herbivore, parasitoid-host, and parasite-host where the trophic level occupied by a species is not body size-dependent. Optimal diet choices are still expected to produce a positive relationship between consumer and resource body sizes if handling time is a function of the consumer:resource body size ratio (Troost et al. 2008). Asymmetric feeding relationships therefore provide simple systems in which to investigate optimal diet choices at the community level.

Models of communities have also focussed on closed “local” communities, where ecological processes are assumed to be the main determinants of community structure. While this framework can be useful to understand the effects of ecological processes, it is increasingly recognized that the concept of “local” communities is often arbitrary; a spatial scale that is local for one species may not be so for another (Cornell and Lawton 1992; Ricklefs 1987; Ricklefs 2008). No community is completely isolated from outside factors such as migration from the larger “regional” pool of species affects the most basic characteristic of “local” communities: species composition (Leibold et al. 2004). Moreover, species found in a given community and feeding relationships among them may be partly determined by their evolutionary history (Cattin et al. 2004; Cavender-Bares et al. 2009; Emerson and Gillespie 2008; Ives and Godfray 2006; Rezende et al. 2009; Vellend 2010; Vitt and Pianka 2005). Patterns of evolutionary history can also be seen in consumer:resource body size relationships (Bersier and Kehrli 2008).

2.5 Phylogenetics

Comparative studies use the natural variation among species to reveal correlations among their traits and with characteristics of their environment. These can be used to derive general patterns or more often to test specific theories in physiology, ecology, or evolution. The comparative method can be especially useful to test hypotheses for which experiments are not possible. Individual species, however, do not constitute independent points in statistical analyses, because closely related species tend to share common traits simply due to their shared ancestry (Felsenstein 1985; Harvey and Pagel 1991; Miles and Dunham 1993). For instance, a comparative study of vertebrates to identify traits that favour flight could reveal a correlation between the ability to fly and the presence of beaks. This correlation is not because each species of bird independently evolved a beak and the capacity to fly, but because bird species inherited a beak (and wings) from their common ancestor.

2.5.1. Phylogenetic signal

Traits that are related to the evolutionary history (phylogeny) of organisms are said to have a phylogenetic signal (Abouheif 1999; Blomberg et al. 2003). A positive phylogenetic signal is characteristic of phylogenetically conserved traits like the presence of beaks in birds. This includes traits such as the diet of animals (Cavender-Bares et al. 2009; Vitt and Pianka 2005; Webb et al. 2002; Wiens and Graham 2005), and body size (Ashton 2004; Blomberg et al. 2003; Freckleton et al. 2002; Kohlsdorf et al. 2008). A negative phylogenetic signal on the other hand, characterizes traits that differ more among closely related species than among groups of more distantly related species is also possible (Abouheif 1999; Blomberg et al. 2003; Pavoine et al. 2008), one example being the beak size in Darwin’s finches, which has diverged a few times through evolution (Sato et al. 1999).

2.5.2. Phylogenetic methods

The use of methods to correct for the phylogenetic dependence of traits is standard practice in comparative studies (Freckleton et al. 2002; Losos 2008; Miles and Dunham 1993). Phylogenetic comparative methods rely on a phylogenetic tree, which is assumed to be correct. In practice, phylogenetic trees are hypotheses derived from molecular, morphological, or even behavioural traits. Large errors in phylogenetic trees can lead to erroneous results. The use of correct but incomplete phylogenetic information is better than using no phylogenetic information at all, which implicitly assumes that all species diverged simultaneously from a common ancestor (Garland et al. 2005). Phylogenetic methods can reveal correlations between traits that are independent from phylogeny, but like all correlations do not indicate causality.

Phylogenetic independent contrasts (PIC) was the first method proposed to correct for phylogenetic dependence, and consist in comparing how two traits change at each branching event in the phylogeny (Felsenstein 1985). Phylogenetic independent contrasts have been shown to be a special case of generalized least square regressions. Generalized least square regressions specify a covariance that has the advantage of following the same framework as linear models. This facilitates the analysis of complex models that can include multiple covariates and interactions, continuous and discrete data, and partly unresolved phylogenies (Paradis 2006). The versatility of GLS is that it follows the same framework as linear models, but specifies a covariance matrix between species as a function of their phylogenetic proximity. Other methods exist which suit different types of data or problems (Miles and Dunham 1993).

In most phylogenetic analyses, a given set of traits is simply measured in a group of organisms and their phylogeny is used in the analysis. In the case of traits that are characteristic of species interactions, however, a single phylogeny may not suffice (Ives and Godfray 2006). Foraging traits such as attack rate or handling time are the outcome of the behavioural interaction between consumers and their resource and may be related to the phylogeny of both protagonists. Comparative studies of foraging traits have attributed these traits to the consumers and accounted for the phylogeny of consumers only (Goss-Custard et al. 2006; Rezende et al. 2009). Ives and colleagues (2006) have elaborated a bipartite model to account for the phylogenies of consumers and resources, when the two belong to clearly defined trophic levels as found in asymmetric food webs. This is also useful for studies of community ecology, where phylogenetic patterns in species interactions are used to support diverse ecological or evolutionary processes (Cavender-Bares et al. 2009; Webb et al. 2002; Webb et al. 2006; Weiblen et al. 2006)

2.6 Aphid-parasitoid interactions

2.6.1. Aphids

Aphids (Hemiptera, Aphididae) are small plant-sucking insects colonizing over 3000 species of herbaceous plants and shrubs (Blackman and Eastop 2006a) and many more are found on trees (Blackman and Eastop 1994). Many aphids specialize on few species of plants and plant selection is normally through the use of chemical cues (Pickett et al. 1992). All aphids go through at least one stage of parthenogenetic reproduction, which can be associated with a change in host plant. Aphids have different morphs associated to their reproductive cycle, seasons, or dispersal, which vary among species (Dixon 1977; Lambers 1966). Generally, at the beginning of the season, eggs hatch into winged fundatrix that seek a host plant where it will give birth to wingless apterae. The latter can reproduce parthenogenetically for many generations and form very large colonies of clone-mates (Loxdale 2008), after which different morphs lead to winged sexual morphs. The sexual forms usually occur at the fall and produce overwintering eggs. All morphs are viviparous except for the sexual morphs. During generations of apterae, poor plant quality, crowding or pseudo-crowding effects caused by the disturbance from natural enemies can produce winged alatae morphs that disperse to new host plants (Braendle et al. 2006; Müller et al. 2001). Nearly all aphids go through four nymphal instars before reaching the adult stage. A few species of gall aphids are eusocial and produce sterile soldier castes (Foster 2002; Stern and Foster 1996; Stern et al. 1996; Wool 2004). The large colonies of clone-mates constitute an abundant resource for many predators and parasitoids (natural enemies), including coccinellids, lacewings, and aphidiine parasitoids. Defences of aphids against natural enemies include escaping (Dixon 1958), fighting back (Gross 1993), or the use of cornicle secretions (Pickett and Griffiths 1980; Wientjens et al. 1973), which contain an alarm pheromone (Pickett and Griffiths 1980; Wientjens et al. 1973) and can also physically hamper an attacker (Edwards 1966). Some aphids are also attended by ants that protect them from natural enemies. Ant-attended aphids have reduced defence responses (Mondor et al. 2002). Aphids also have some immunological defences that can kill the egg or larvae of a parasitoid inside their body (Vinson and Iwantsch 1980).

2.6.1. Aphid parasitoids

Females of *A. colemani* lay their eggs inside (endoparasitoid) aphids. The eggs hatch and the larvae feed off the living host (koinobiont parasitoid), killing it by the fourth and last instar. The larvae then weave a protective cocoon inside the exoskeleton of the aphid before reaching the adult stage. Unfertilized eggs emerge as males and fertilized eggs emerge as females (haplodiploid) that will seek new hosts shortly after emergence (Starý 1988).

Aphidiine parasitoids (Hymenoptera, Braconidae, Aphidiinae) are the principal parasitoids of aphids and about 400 species have been described (Starý 1988), and new species are discovered regularly (e.g. Kavallieratos et al. 2006; Pike and Starý 1996; Starý and Zuparko 1995; Tomanović and Kavallieratos 2002). They are koinobiont endoparasitoids so females oviposit their eggs inside living hosts, which can continue feeding during the initial development of the parasitoid larvae (Gauld 1988). Like other parasitoids, they cause the death of their hosts before reaching the adult stage (Reuter 1913; Godfray 1994). Aphidiine parasitoids are also solitary parasitoids, so only one parasitoid can emerge from each host even if multiple eggs have been laid (van Alphen and Visser 1990). The quality of the host affects the body size of emerging parasitoids and traits correlated to body size such as fecundity (Barrette et al. 2009). Host quality varies with host species (Nicol and Mackauer 1999; Ode et al. 2005), body size (Jenner and Kuhlmann 2006), developmental stage (Brodeur et al. 1996; He et al. 2005), the presence of another parasitoid immature (Persad and Hoy 2003; Silva et al. 2008b) or bacterial symbionts (Cheng et al. 2010; Miao et al. 2004; Stadler and Mackauer 1996). Because the number and quality of hosts parasitized approximates reproductive success, the fitness of aphidiine parasitoids should be tightly linked to the females' host choice decisions and host foraging behaviour. Host finding and handling should therefore be under strong selection pressure, which makes them ideal to study host foraging behaviour (Godfray 1994; Godfray and Shimada 1999). Female aphidiine parasitoids emerge with mature eggs and produce more through their life (Starý 1988) to achieve a lifetime fecundity reaching 200-500 eggs (Barrette et al. 2009; Cloutier et al. 2000; Colinet et al. 2005; Giri et al. 1982; Shirota et al. 1983; Silva et al. 2008b; Torres et al. 2007) and even 1770 eggs (Mackauer 1983). For aphidiine parasitoids in the field where longevity may be short and available foraging time may be restrained by unfavourable weather conditions (Weisser et al. 1997), reproductive success can be limited by time constraints as well as egg load (Heimpel et al. 1998; Minkenberg et al. 1992; Rosenheim 1999; Rosenheim et al. 2008).

Host selection by aphidiine parasitoids can be broken down to steps (Starý 1964; Vinson 1976) going from habitat location, host location usually through plant volatiles, some of which are induced by feeding aphid (Du et al. 1998; Girling et al. 2006; Liu et al. 2001; Vet and Dicke 1992), to host acceptance influenced by host quality (He et al. 2005; van Lenteren and Bakker 1975). In addition to host quality, the cost of parasitism is also important since aphids can defend themselves behaviourally (Brodeur et al. 1996; Gross 1993; Walker & Hoy 2003;

Godfray 1994). Recent studies also suggest that phylogeny plays a role in host choices (Desneux et al. 2009; Silva et al. 2008a)

Figure 2.6.1 *Aphidius colemani* exploiting a patch of first instar and adult *Myzus persicae* aphids (© Gi-Mick Wu 2010)

Over 400 species of aphids feed on agricultural crops and cause considerable economic damage through feeding and disease transmission (Blackman and Eastop 2000). Understanding how aphids and parasitoids interact can be used to improve the use of parasitoids to control aphid populations (Wei et al. 2005) and to avoid undesirable consequences for non-target species (van Veen et al. 2006). Aphidiine parasitoids such as *A. colemani* (Figure 2.6.1) are already used successfully to control aphids in greenhouses especially (van Lenteren 2006).

PREFACE TO CHAPTER 3

This is the first of three chapters (3-5) studying aphid parasitoids at the individual level, and investigates body size as a determinant of handling time. It begins by investigating how handling time varies with natural variation in parasitoid and host body sizes using the parasitoid *Aphidius colemani* and the aphid host *Myzus persicae* as a model system. It then uses the developmental temperature of immature parasitoids to manipulate adult body size and test its effect on the handling capacity of adult parasitoids.

CHAPTER 3

TEMPERATURE AFFECTS HANDLING TIME THROUGH BODY SIZE-MEDIATED EFFECTS

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3.1 Abstract

1. The body size of ectotherms increases with decreasing developmental temperature (temperature-size rule). Because the body size of consumers relative to their resources often affects their foraging efficacy, we hypothesize that increasing developmental temperature will increase the handling time of ectothermic foragers.
2. We conducted laboratory experiments using an aphid-parasitoid model system. We first determined the extent to which naturally occurring body size variations in the parasitoid and its host (different instars) affect handling time. We then tested the effect of rearing immature parasitoids at 15ºC and 25ºC on the handing time of adults attacking first instar and adult aphids.
3. As expected, the parasitoids' handling time increased together with aphid instar. Furthermore, the parasitoids' handling time decreased with parasitoid body size, but only when exploiting adult aphids.
4. Developmental temperature had the expected effect on parasitoids: those reared at 25ºC were smaller than those reared at 15ºC. Parasitoids reared at 25ºC (small) also took longer to handle adult aphids than first instar aphids, but those reared at 15ºC (large) had similar handling times for both first instar and adult aphids.
5. Our results showed that developmental temperature can influence some aspects of foraging behaviour in a simple way; through body size-mediated effects. Moreover, both the temperature-size rule and the relationship between handling time and body size ratio are quite widespread so this result likely applies to many ectothermic foragers.

Keywords: foraging; handling time; relative body size; temperature-size rule

3.2 Introduction

Temperature modulates the foraging behaviour of ectothermic animals, because their metabolic rate follows ambient temperature as shown in fishes (Clarke and Johnston 1999), reptiles (Stevenson et al. 1985), and insects (Nespolo et al. 2003) for instance. Variations in ambient temperature have a direct effect on locomotion speed, activity level (Byström et al. 2006; Stevenson et al. 1985; Vogt et al. 2003), and foraging behaviour (Avery and Mynott 1990; Langer et al. 2004; Vincent and Mori 2008) of ectotherms. Furthermore, temperature experienced during development of immature forms could affect the foraging behaviour of the adults indirectly. The temperature-size rule describes the inverse relationship between the developmental temperature and adult body size of ectotherms (Atkinson 1994; Walters and Hassall 2006). The effect of developmental temperature on adult body size is associated with changes in other life history traits such as maturation rate, fecundity and longevity (Colinet et al. 2007; van der Have and de Jong 1996). Temperature-induced changes in body size can also have implications for the foraging behaviour of animals, as body size often affects the ability of animals to handle prey or hosts. This may contribute to the understanding of mechanisms by which temperature affects the seasonal or biogeographical distribution of ectothermic animals. Few studies have addressed the ecological consequences of the temperature-size rule for foraging ectotherms.

When exploiting a resource, foragers incur a handling time, which consists of pursuing, subduing and killing their prey or hosts (Sih and Christensen 2001). Handling time, a key parameter characterizing the value of a resource, affects the diet choice of optimally foraging animals (Charnov 1976a; Stephens and Krebs 1986). Because handling time decreases with increasing forager body size, relative to its resource (Griffiths 1980b), we hypothesized that developmental temperature affects the foraging behaviour of ectotherms through body size-mediated effects. We predicted that handling time should increase with developmental temperature (decreasing adult body size).

We used aphid parasitoids (Hymenoptera, Braconidae, Aphidiinae) to test the prediction of our hypothesis. Female aphidiin parasitoids reproduce by laying an egg in a living aphid, which later serves as food for the developing offspring. Because each offspring develops from a single aphid host, its adult size is largely determined by the amount of resources inside its host (Cloutier et al. 2000; Liu 1985), even though these parasitoids are koinobionts (their hosts continue to feed and grow after being parasitized; Gauld 1988). The size of parasitoids can vary considerably in nature, because females usually attack multigenerational colonies of aphids containing individuals of varying size (Cohen et al. 2005). Moreover, immature developmental temperature has been shown to affect the body size of adult parasitoids (Colinet et al. 2007). Female parasitoids incur handling cost when attempting to subdue their hosts or cope with their hosts' defense behaviors (Barrette et al. 2009). Aphidiinae are therefore ideal to test the effect of developmental temperature on adult handling efficiency.

We conducted laboratory experiments using the aphid parasitoid, *Aphidius colemani* (Viereck) (Hymenoptera, Braconidae, Aphidiinae) and one of its common hosts, *Myzus persicae* (Sulzer) (Hemiptera, Aphididae). A first experiment examined the effect of parasitoid body size on handling time for aphids of different sizes (instars). A second experiment tested the effect of developmental temperature on the handling time of parasitoids attacking first instar and adult aphids.

3.3 Methods

3.3.1 Insect cultures

Insect cultures were maintained in the laboratory on sweet pepper (*Capsicum annuum* L.) under a 16 h photoperiod at 20±1ºC and 60-65% relative humidity. For Experiment 1, *M. persicae* was obtained from the greenhouses of the Horticultural Research and Development Centre (Saint-Jean-sur-Richelieu, QC), and *A. colemani* was provided by Koppert Canada. For Experiment 2, *M. persicae* was collected at Louvain-la-Neuve, Belgium, and *A. colemani* was provided by Biobest Belgium. Aphids of all four larval instars as well as adults were obtained by producing cohorts of aphids aged 1, 3, 5, 7, and 9 days, respectively, under the same conditions as insect cultures. For Experiment 1, cohorts were initiated by allowing adult apterous females to reproduce during 8 h on an excised pepper leaf. Leaves were kept fresh by dipping their petiole in a 1.5 ml Eppendorf vial containing distilled water and replaced every 3-4 d. For Experiment 2, aphid cohorts were maintained on an artificial medium held between two layers of stretched Parafilm (Cambier et al. 2001). Parasitoids used in the experiments were obtained by allowing female parasitoids to parasitize third instar aphids during 4 h. Parasitized aphids were kept on an excised pepper leaf in a Petri dish for Experiment 1, and on an artificial medium for Experiment 2. Aphids that did not form a mummy were removed from the Petri dish prior to the emergence of parasitoids. Emerging parasitoids were kept in the Petri dish 24-48 h to allow mating to occur, but kept naïve (no exposure to aphids).

3.3.2 Experiment 1: Parasitoid body size vs handling time

We measured handling time of parasitoids attacking four larval instars and adult aphids. Prior to trials, 20-30 aphids of a given instar were placed in a

Petri dish and were given 1-3 h to settle on five rectangular pieces of pepper leaves measuring 0.8-1.0 cm^2. At the start of a trial, one piece of leaf containing three aphids (superfluous individuals being discarded) was gently placed in the center of a Plexiglas arena (20 x 15 x 5 mm). A single parasitoid was then introduced in the arena, which was covered by a glass microscope slide. Parasitoids that did not initiate an attack after 5 min were discarded. A trial was ended when 5 min had elapsed without any attacks. For each aphid instar, 13-17 replicates were conducted for a total of 73 trials.

3.3.3 Experiment 2: Developmental temperature vs handling time

The effect of developmental temperature on the handling time of parasitoids was determined using a two-by-two experimental design. Parasitoids were reared at either 15ºC or 25ºC and were next tested with either first larval instar or adult aphids. Foraging trials were conducted as in Experiment 1, but in a glass arena measuring 26 x 20 x 5 mm. For each treatment combination, parasitoids were used only once and 10-11 replicates were conducted for a total of 41 trials.

3.3.4 Handling time and body size measurements

Video-recordings of trials were digitized and analyzed using The Observer 5 video-pro software (Noldus Information Technology, Leesburg, VA). In order to measure the handling times of ovipositions_accurately, videos were viewed 1/5 the normal speed during attacks, and the following behaviours were recorded: 1) stationary: remaining immobile without any noticeable activity; 2) walk: walking in the arena; 3) contact: touching an aphid using antennae; 4) sting: contact of an aphid using the ovipositor, accompanied by the elongation of the abdomen and backward position of antennae; and 5) grooming: rubbing of antennae, head or abdomen using legs or mandibles. Handling time of parasitoids was defined as the time from contact of a host to the end of a sting including any grooming time that followed stinging, unless it was separated by another contact. Handling times of all interactions in a trial were averaged for each parasitoid.

Each parasitoid was used only once, then sacrificed and mounted on a microscope slide to be photographed (10X) and measured. Total body length from the base of the antenna to the end of the abdomen was obtained from digital images using Image-Pro version 5. In experiment 2, only hind tibia length was measured, but body length and hind tibia length are highly correlated in *A. colemani* (r=0.92, n=73; unpublished data).

3.3.5 Statistical Analyses

Generalized linear models were used to analyse handing times with Gamma-distributed error terms (log link function), because of the nature of the data (short time intervals). The statistical model also fitted the data better when assuming a Gamma distribution rather than a normal distribution, as supported by its smaller AIC value (Akaike 1971); ∆AIC >30 (Burnham and Anderson 2002). Handling time was analysed as a function of ranked aphid instars (1, 2, 3, 4, 5) and parasitoid body length in Experiment 1, and as a function of ranked aphid instars (1 & 5) and parasitoid rearing temperatures (25ºC, 15ºC) in Experiment 2. Interaction terms were included in each model and post-hoc tests were conducted for each factor level when the interaction was significant ($\alpha < 0.05$). To obtain robust tests of significance, p-values were obtained from 10000 bootstrap samples. All analyses were performed using R version 2.9.0 (R Development Core Team 2009).

3.4 Results

3.4.1 Experiment 1: Parasitoid body size vs handling time

Parasitoid females ranged from 1.73-2.46 mm in body length and the average host handling time ranged from 1.1-11.4 s. Parasitoid handling time increased significantly with aphid instar ($p<0.0001$; Figure 3.4.1). Post hoc pairwise comparisons revealed that handling time differed significantly between each successive instar (all p's<0.01). There was, however, a significant interaction between aphid instar and parasitoid size ($p=0.0249$). Post-hoc tests by aphid instar revealed that handling time decreased with increasing parasitoid size for attacks on adult aphids only ($p=0.0144$; Figure 3.4.1).

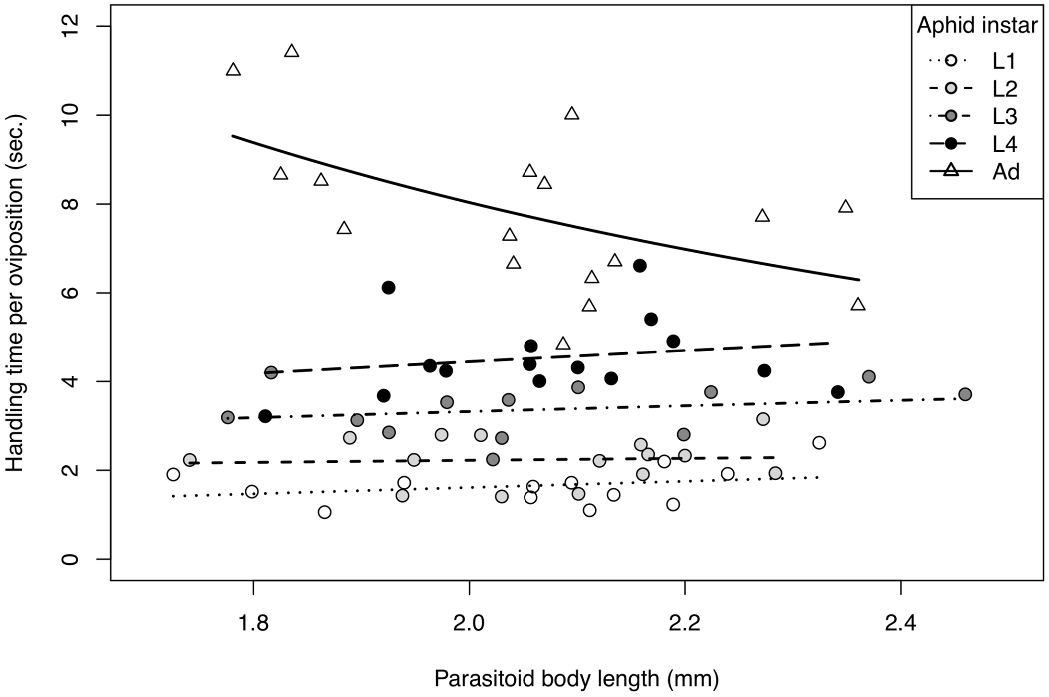

Figure 3.4.1 Handling time of *Aphidius colemani* as a function of parasitoid body length and instar of *Myzus persicae*. Handling time (T_h) decreased significantly with increasing parasitoid body length (BL) for adult aphids only ($T_h = 22.29 \times BL^{-1.472}$).

3.4.2 Experiment 2: Developmental temperature vs handling time

Parasitoid rearing temperature had a significant effect on the size of the adult female parasitoids ($p<0.0001$); the mean (±SE) hind tibia length of parasitoids reared at 15˚C (555±5 μm, n=20) was greater than that of parasitoids reared at 25˚C (525±4 μm, n=21). Handling time of parasitoids was longer when attacking adult aphids than first instar aphids ($p=0.0024$), and there was a significant interaction between parasitoid rearing temperature and aphid instar ($p=0.0463$). Post-hoc tests showed that parasitoids reared at 25ºC took significantly longer to handle adult aphids

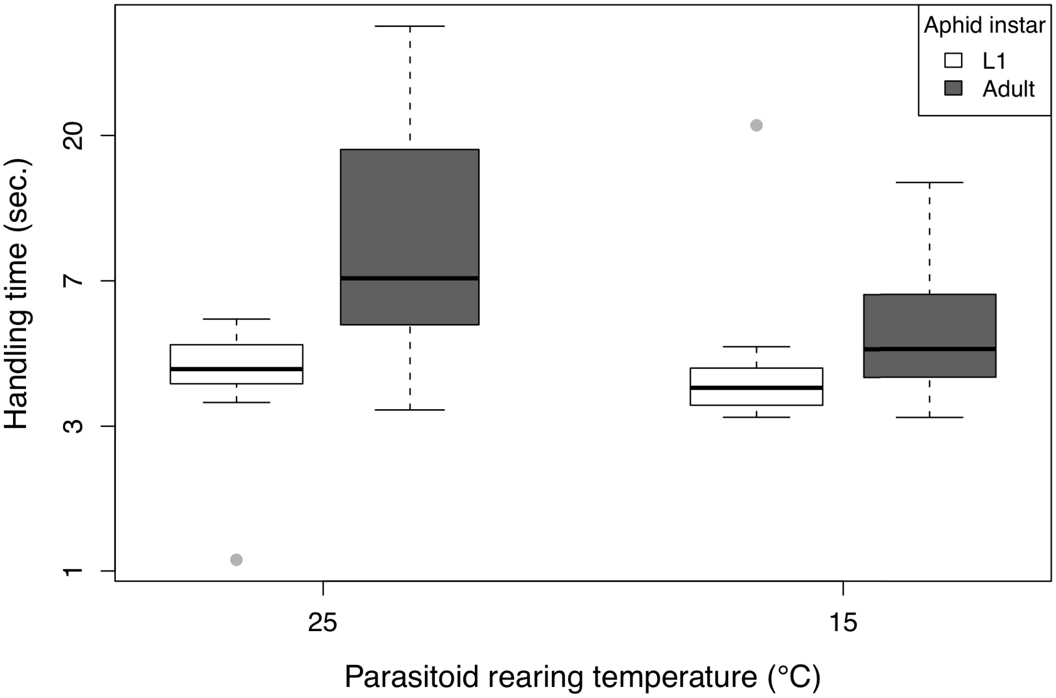

Figure 3.4.2 Handling time (log scale) of *Aphidius colemani* as a function rearing temperature and instar of its host, *Myzus persicae*. Boxes show the interquartile range, in which the horizontal line indicates the median. Vertical bars extend to 1.5 the upper and lower interquartile range, beyond which data are usually considered outliers (dots); removing these two points did not change the significance of the results.

than first instar aphids (p=0.0018), while parasitoids reared at 15ºC had similar handling times for both first instar and adult aphids (p=0.3792; Figure 3.4.2).

3.5 Discussion

Our results are consistent with the hypothesis that the developmental temperature of parasitoids affects their handling time (when adults) through body size-mediated effects. In addition, the effects of both temperature and naturally occurring variations in parasitoid body size on handling time were dependent on the size of the host attacked. We discuss how temperature changes may affect the foraging behaviour of ectothermic animals.

The results of our two experiments support the hypothesis that the effects of developmental temperature on the foraging of adult parasitoids are mediated by body size. Naturally occurring (Experiment 1) and temperature-induced (Experiment 2) variations in adult parasitoid size had similar effect on handling time. In both cases, there was an interaction between the effects of parasitoid size and aphid size on handling time. The consistency in the results of the two experiments, despite the differences between the origin of insects and protocols, suggests that the relationship between handling time and body size ratio is robust. This result is consistent with those found recently for another aphid parasitoid species, *A. ervi* (Henry et al. 2009), and also in predatory lizards (Cruz-Neto et al. 2001), where the size of the forager affected handling time for large, but not small hosts or prey. For aphid parasitoids, the observed pattern may be partly due to young aphids expressing fewer and less effective defences than older aphids (Chau and Mackauer 2001b; Gerling et al. 1990; Kairo and Murphy 1999a; Walker and Hoy 2003). Small aphids are likely easy to exploit for parasitoids of all sizes, whereas large aphids may be difficult to subdue for small, but not for large parasitoids. Body size-dependent handling time could also explain patterns of parasitism success in small and large parasitoids. In a recent study, large aphids (*Aphis gossypii*) were parasitized in greater proportion when exposed to large rather than small *A. colemani*, whereas small aphids were parasitized in similar proportion by small or large parasitoids (Lykouressis et al. 2009).

Effects of temperature that persist throughout both immature and adult stages of ectotherms may have opposing effects on adult foraging efficacy. In this study, increasing the developmental temperature of immature parasitoids reduced the adult body size and thereby the handling efficiency. This is opposite to effects of ambient temperatures on the handling time of *A. colemani* (Zamani et al. 2006), other parasitoids (Bueno and van Cleave 1997; Enkegaard 1994), and foraging rate of ectotherms in general (Avery and Mynott 1990; Byström et al. 2006). The net effect of temperature on the foraging behaviour of ectotherms may therefore depend on the relative influence of temperature on the immature and adult stages, as suggested by results from other studies on parasitoids. As expected, temperature treatments that were maintained through both the immature and adults stages of the aphid parasitoid *Diaeretiella rapae* had no effect on the overall parasitism success of the wasps (Bernal and Gonzalez 1997). In another study, reductions in temperature applied only to the immature stage of *A. colemani* increased realized fecundity as expected from body size-mediated effects, except for the coldest temperature, which approaches the minimum temperature threshold (Colinet et al. 2007).

For short-lived ectotherms such as aphid parasitoids, seasonal differences or long term changes in temperature may have weak effects on handling time. On the other hand, variability in temperatures associated with climatic zones, with La Niña (Smith and Sardeshmukh 2000) or with global climate change (Meehl et al. 2007) may have a greater impact. Alternating cold and hot spells will create conditions similar to Experiment

2, where: a) temperature during immature and adult stages differ; and b) parasitoids and hosts with different generation times experience different temperatures during their development. This will be important in determining the effects of temperature variability on the foraging behavior of insects. Daily temperature cycles will tend to result in mean developmental temperatures lower than foraging temperatures for these diurnal insects.

Temperature-induced variations in parasitoid body size and the consequences on handling may also have implications for the mass production and release of biological control agents, for which the temperatures experienced by developing immatures and by foraging adults can be decoupled. For instance, parasitoids could be reared at low temperatures to produce large individuals, and then be released in a warm greenhouse to increase their activity level.

Handling time is expected to influence diet choice (Sih and Christensen 2001), patch residence time (Hassell and Southwood 1978), population dynamics (Henry et al. 2009; Schreiber and Vejdani 2006), and food web structure (Petchey et al. 2008), so that our results have implications for these aspects of foraging behaviour as well. While handling time of Aphidiinae is typically short, it represents a considerable lost opportunity cost, because aphids emit an alarm pheromone that quickly disperses the colony when attacked (Dawson et al. 1982; Montgomery and Nault 1977; XiangYu et al. 2002). Hence, an alerted colony quickly depletes itself while the parasitoid is engaged handling a host.

Body size-mediated effects of developmental temperature on foraging should apply to a broad range of ectotherms, because of the universality of the temperature-size rule (Atkinson 1994; Walters and Hassall 2006) and body size effects on foraging animals (Cruz-Neto et al. 2001). In order to predict the overall consequences of developmental temperature on the fitness of ectotherms, however, other life history traits such as development time and immature survival that are also modulated by temperature must be considered (Angilletta et al. 2004; Hance et al. 2007; Sampaio et al. 2007).

PREFACE TO CHAPTER 4

The previous chapter showed that the relative body size of parasitoids and their aphid hosts affect handling time, and how this could mediate effects of temperature on handling time. Chapter 4 investigates the effects of an adaptation specific to aphids, cornicle secretions, on the handling time of parasitoids. Here, another aphid parasitoid, *Aphidius rhopalosiphi*, and its host *Sitobion avenae* are used as a model system.

CHAPTER 4

ALTRUISTIC DEFENCE BEHAVIOURS IN APHIDS

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4.1 Abstract

Background

Altruistic anti-predatory behaviours pose an evolutionary problem because they are costly to the actor and beneficial to the recipients. Altruistic behaviours can evolve through indirect fitness benefits when directed toward kin. The altruistic nature of anti-predatory behaviours is often difficult to establish because the actor can obtain direct fitness benefits, or the behaviour could result from selfish coercion by others, especially in eusocial animals. Non-eusocial parthenogenetically reproducing aphids form colonies of clone-mates, which are ideal to test the altruistic nature of anti-predatory defence behaviours. Many aphids release cornicle secretions when attacked by natural enemies such as parasitoids. These secretions contain an alarm pheromone that alerts neighbours (clone-mates) of danger, thereby providing indirect fitness benefits to the actor. However, contact with cornicle secretions also hampers an attacker and could provide direct fitness to the actor.

Results

We tested the hypothesis that cornicle secretions are altruistic by assessing direct and indirect fitness consequences of smearing cornicle secretions onto an attacker, and by manipulating the number of clone-mates that could benefit from the behaviour. We observed parasitoids, *Aphidius rhopalosiphi*, foraging singly in patches of the cereal aphid *Sitobion avenae* of varied patch size (2, 6, and 12 aphids). Aphids that smeared parasitoids did not benefit from a reduced probability of parasitism, or increase the parasitoids' handling time. Smeared parasitoids, however, spent proportionately more time grooming and less time foraging, which resulted in a decreased host-encounter and oviposition rate within the host patch. In addition, individual smearing rate increased with the number of clone-mates in the colony.

Conclusions

Cornicle secretions of aphids were altruistic against parasitoids, as they provided no direct fitness benefits to secretion-releasing individuals, only indirect fitness benefits through neighbouring clone-mates. Moreover, the use of cornicle secretions was consistent with their altruistic nature, because the occurrence of this behaviour increased with the size of indirect fitness benefits, the number of clone-mates that can benefit. This study provides evidence for a case of kin-directed altruistic defence outside eusocial animals.

4.2 Background

4.2.1 Altruistic defences

The adaptive value of most anti-predatory behaviours is quite intuitive, as they aim to lower mortality of the actor due to predation. However, behaviours such as alarm signalling in birds and mammals (Caro 2005), predator inspection by fish (Brown and Godin 1999; Pitcher 1992) or aggressive defences by worker honeybees, pose an evolutionary challenge, because the cost of these behaviours is born by the actor, while other individuals (recipients) benefit from them. In some cases, the actor also obtains selfish benefits that are enough to offset the costs of the behaviour, so that benefits to others may be incidental (mutual benefit or weak altruism) (West et al. 2007b; Wilson 1990). In more extreme cases of altruism, the actor incurs a net fitness cost and the behaviour can evolve through indirect fitness benefits if it is preferentially directed toward individuals (usually kin) who share the same genes (Hamilton 1963; Maynard-Smith 1964; West et al. 2007b). In this paper, we refer to altruism as the latter, more extreme form of altruism.

In animals living in groups, many anti-predatory behaviours benefit individuals other than the actor, but few have been shown to be purely altruistic. Alarm signals, for instance, warn conspecifics of the presence of potential predators (Caro 2005; Pasteels et al. 1983; Smith 1992), but may be used selfishly to manipulate other group members (Charnov and Krebs 1975) or to inform the predator that the actor is more difficult to catch than other individuals (Searcy and Nowicki 2005; Shelley and Blumstein 2005; Woodland et al. 1980). Likewise, predator inspection increases exposure to predation for the benefit of the group (Dugatkin 1997), but the actor may obtain better information and consequently escape attacks more easily than other individuals (Brown and Godin 1999; Fishman 1999). Aggressive defences are clearly costly to the actor and benefit recipients in the form of protection or an opportunity to escape (Caro 2005). Aggressive attacks, and other seemingly altruistic anti-predatory behaviours, can increase mating success of the actor (Tessman 1995), and survival of potential mates (Krams et al. 2006) or direct descendants (Montgomerie and Weatherhead 1988) of the actor. Because mating success and the survival of direct offspring (parental care) provide the actor with direct fitness benefits, these behaviours can be qualified as mutually benefiting rather than altruistic (West et al. 2007b). In the case of eusocial animals, anti-predatory behaviours are performed by non-reproductive castes such as worker or soldier ants (Hölldobler and Wilson 1990). While these behaviours clearly benefit the reproductive queen(s) and not the actor, they may be the result of selfish control by the queen or by other individuals in the colonies (Gadagkar 2009; Ratnieks and Wenseleers 2008).

Parthenogenetically reproducing animals that form groups of clone-mates such as aphids (Hemiptera: Aphididae) (Dixon 1977; Loxdale 2008) provide the ideal system to test kin-selection of altruistic behaviours used against predators or insect parasitoids. Because these aphids are not eusocial, selfish manipulation by queens or workers is not a confounding factor. Additionally, other studies suggest that altruistic defences can evolve in aphids. For instance, pea aphids (*Acyrthosiphon pisum*) that have been parasitized tend to drop off their plant and increase their chances of dying, thereby decreasing parasitoid load for the following generations of aphids (McAllister and Roitberg 1987; McAllister et al. 1990). However, non-altruistic interpretations of this suicidal behaviour have been proposed (Latta 1987; Tomlinson 1987).

4.2.2 Aphid cornicle secretions

Group living confers aphids with many anti-predatory benefits (Caro 2005; Gross 1993; Pusey 2005). For instance, colonies of aphids create a dilution effect (Lucas and Brodeur 2001), which can also be enhanced with decoys by leaving empty exoskeletons after moulting (Muratori et al. 2008) or by remaining near dead aphids (Fievet et al. 2009). In addition, most species of aphids also possess a pair of cornicles, which are projections that stick out of their abdomen (Blackman and Eastop 2006b). When attacked by an enemy (i.e., a predator or an insect parasitoid), aphids can release sticky secretions (Edwards 1966) that contain an alarm pheromone (Callow et al. 1973) from the tips of these cornicles. The alarm pheromone of aphids, (E)-*β*-farnesene, elicits defensive or escape responses in neighbouring aphids (Dawson et al. 1982; Montgomery and Nault 1977; XiangYu et al. 2002) and increases their survival (Mondor and Roitberg 2004). Because neighbours (recipients) are often clone-mates, the alarm function provides indirect fitness benefits to the aphid releasing the secretions (the actor). Furthermore, cornicle secretions are released in a super-cooled liquid form, which hardens upon contact with an object such as an enemy (Edwards 1966). These hardening secretions hinder enemies and could reduce the risk of predation or parasitism for the aphid releasing them (Dixon 1958), thereby having a direct fitness benefit. A predator or parasitoid could also be temporarily or permanently incapacitated by the hardened secretions and unable to attack nearby aphids (Butler and O'Neil 2006) or may decide to leave the colony prematurely (Outreman et al. 2005). This would provide the aphid releasing the secretions an indirect fitness benefit through increased survival of clone-mates.

Cornicle secretions are mostly composed of triglycerides (Callow et al. 1973) and therefore costly to produce for sap-feeding aphids, which lack lipids in their diet. The release of cornicle secretions reduces the amount of lipid available for development (Byers 2005), reproduction or dispersal (Dillwith et al. 1993). Releasing even a single droplet can delay or reduce reproduction of aphids, especially when doing so before attaining maturity (Mondor and Roitberg 2003). Furthermore, releasing cornicle secretions can have an ecological cost as the volatiles contained in them may attract species of predators (Outreman et al. 2010; Verheggen et al. 2009) and parasitoids (Du et al. 1998; Micha and Wyss 1996).

Whereas the alarm function of cornicle secretions clearly provides indirect fitness benefits, the fitness consequences of their mechanical function (smearing) remains unclear. Determining whether the mechanical function of cornicle secretions provides a direct fitness benefit to an aphid is required to establish whether cornicle secretions can be considered altruistic as a whole.

4.2.1 Objectives

In this study, we tested the hypothesis that the release of cornicle secretions by an aphid is altruistic, using the parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) foraging in colonies of grain aphids, *Sitobion avenae* (Hemiptera: Aphididae) in laboratory experiments. We tested for the presence of a direct fitness benefit of cornicle secretions by investigating the effects of smearing on the success of parasitoid attacks. We tested for the presence of indirect fitness benefits by determining whether a parasitoid's rate of oviposition within a colony varied with the frequency of smearing events in that colony. As the number of clone-mates that can benefit from an aphid's release of cornicle droplets increases, the indirect fitness benefit should also increase. Finally, we tested the prediction that cornicle secretions are released more readily when the indirect fitness benefit is greater by manipulating the number of clone-mates in the colony.

4.3 Methods

4.3.1 Study system

Sitobion avenae, a common aphid of cereal crops, is parasitized by the solitary parasitoid *A. rhopalosiphi*. When under attack, this aphid releases cornicle secretions, to which conspecifics respond by waving antennae, ceasing to feed, or dispersing (Dawson et al. 1982; Wientjens et al. 1973). Specimens of the aphid and its parasitoid were collected from wheat (*Triticum aestivum*) fields in Rennes, France, and reared in the laboratory. The rearing of aphids was initiated from a single clonal individual, such that relatedness was at its highest. Colonies were kept at 20˚C, with a relative humidity of 70±10% and a 16L:8D photoperiod. Second instar aphids and one day old mated female wasps were used in the experiment. Prior to testing, female wasps were allowed to gain experience by ovipositing into three aphids on a single wheat leaf.

4.3.2 Laboratory experiment

Female parasitoids (n=50) were allowed to forage individually in a glass cage (40 x 30 x 50cm) containing eight single-leafed wheat plants (15cm tall). Three hours before behavioural observations, six of the eight plants were inoculated with 2, 6, or 12 clone-mates in equal proportions (two plants per aphid density). The two remaining plants were not infested. This range of patch density is commonly encountered by the parasitoid females under natural conditions (Dedryver 1987). Empty patches (0 aphids) were not included in analyses, as no aphid-parasitoid interactions could be observed in them. Each parasitoid was introduced individually at the centre of the cage and observed continuously until it had either visited all eight patches (i.e., the eight wheat plants) or two hours had elapsed. For each patch visit, the observer recorded the number of aphids in the patch, as well as the behaviour of the parasitoid on that patch. Behaviours were defined as *searching* (walking on the plant), *stationary* (immobile), *grooming* (often to remove cornicle secretions), *oviposition* (encountering and stinging a host), and *rejection* (encountering a host without stinging). Stinging was assumed to result in oviposition, because *A. rhopalosiphi* cannot distinguish freshly parasitized from unparasitized hosts (Outreman et al. 2001). Smearing of the parasitoid with cornicle secretions during an attack was also recorded. The timing of events was recorded with a 0.1s precision using “The Observer 3.0” (Noldus Information Technology, Wageningen, The Netherlands).

4.3.3 Statistical Analyses

We assessed the presence of direct fitness benefits to the actor by determining whether smearing during an attack was associated with a lower probability of being stung. When aphids were stung, we also tested the effect of smearing during an attack on the parasitoid’s handling time, because longer handling times may increase the probability that a parasitoid will give up or be interrupted. For each analysis, we included patch density (2, 6, 12 aphids/patch) and timing of attacks (time spent in the patch) as covariates.

We assessed the potential indirect fitness benefits of smearing by measuring the effects of smearing frequency on the parasitoid’s oviposition rate (number of ovipositions offset by patch time) in the patch. We also included aphid density as a covariate. To understand how smearing affects oviposition rate, we tested the effect of smearing frequency on components of the parasitoid’s oviposition rate: encounter rate (number of encounters offset by patch time); the outcome of host encounters (oviposition vs. rejection); average handling time of ovipositions (time spent in oviposition offset by the number of ovipositions); and the proportion of time spent foraging (searching for and handling hosts), grooming, and stationary (duration of behaviours offset by patch time). Aphid density was included as a covariate. Finally, we evaluated the effect of aphid density on smearing rate (smearing frequency offset by patch time). The number of host encounters was included as a covariate to account for the simple effect of increased aphid abundance. We included all second order interaction terms in statistical analyses, but present only significant interactions.

We used generalized estimating equations to account for the different families of distributions of the dependent variables and the multiple observations per subject (Zeger and Liang 1986). The statistical analyses used binomial (logit link function), Poisson (log link function), and Gamma (log link function) error terms for the outcome of encounters (oviposition vs. rejection), frequencies of behaviours, and handling times, respectively. We specified “independence” (no correlation) as the working correlation structure for within-subject observations, because the true correlation structure was unknown (Pan 2001). We tested the robustness of this specification by comparing the fit of each statistical model using two other common working correlation structures: “exchangeable” (fixed correlation within individual) and “auto-regressive (1)” (correlation increasing with proximity between observations) using the QIC information criterion (Pan 2001). The independence working correlation structure almost always produced the best fit. When a different correlation structure produced the best fit, results of the analyses did not differ using the different correlation structure.

We included only data from patch visits in which the parasitoid oviposited in at least one aphid in order to reduce any effects of patch depletion. When analysing patch data, we excluded those with three or four occurrences of smearing, because such frequencies were extremely rare (5 and 1 patch visits, respectively) and occurred only in patches of 12 aphids. Analyses were conducted using the package “geepack” version

1.0-16 (Yan 2002; Yan and Fine 2004) in R version 2.9.0 (R Development Core Team 2009).

4.4 Results

We gathered over 66 hours of observations for the 50 foraging parasitoids. Observations consisted of 326 patch visits that included ovipositions. Patch visits lasted approximately 6.7 minutes (median). We observed a total of 6019 encounters of which 1837 (31%) resulted in oviposition, and 132 (2%) included smearing of the parasitoid with cornicle secretions.

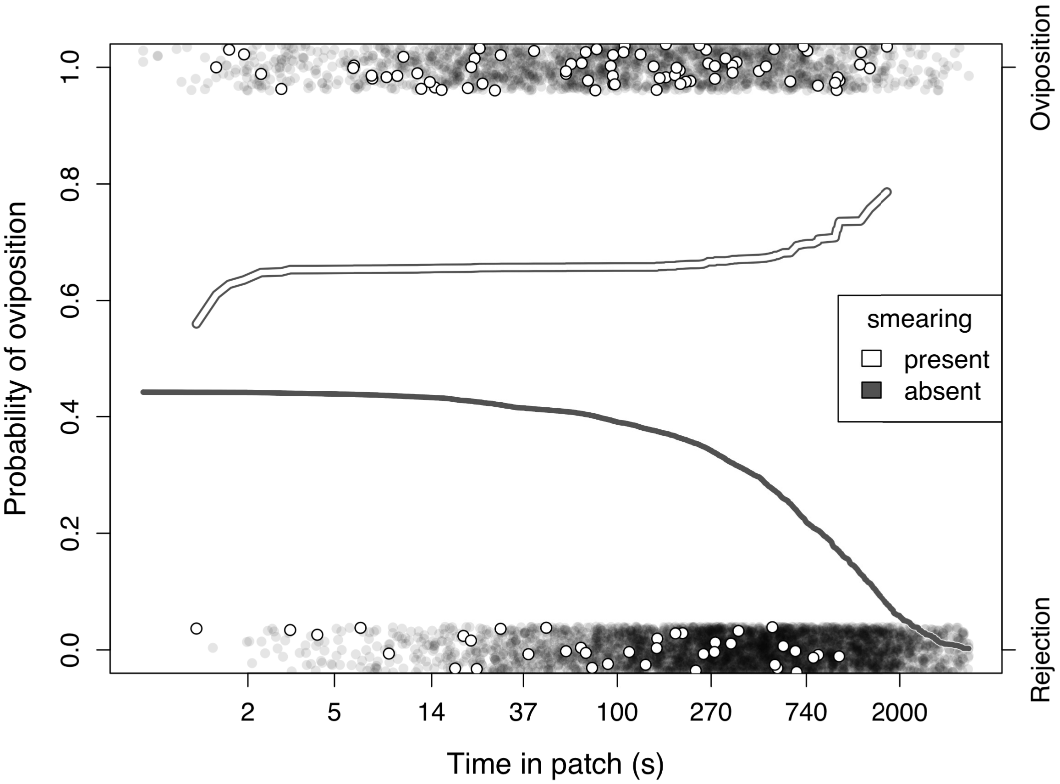

Figure 4.4.1 Encounters resulted in oviposition more frequently in the presence of smearing (white), than in its absence (grey). Lines show the fitted probabilities of oviposition and dots show the outcome of individual encounters (oviposition vs. rejection), as a function of the timing of events.

4.4.1 Direct fitness benefits of smearing

Smearing did not reduce an aphid's probability of being the victim of an oviposition (Wald = 2.64, df = 1, p = 0.10). On the contrary, aphids that smeared their attacker had a higher probability of being a victim of oviposition during the later part of patch visits (interaction: smearing × timing of events, Wald = 8.59, df = 1, p = 0.0034; Figure 4.4.1). Aphid density did not affect the outcome of individual encounters (Wald = 1.33, df = 1, p = 0.25). Handling time was similar for ovipositions during which

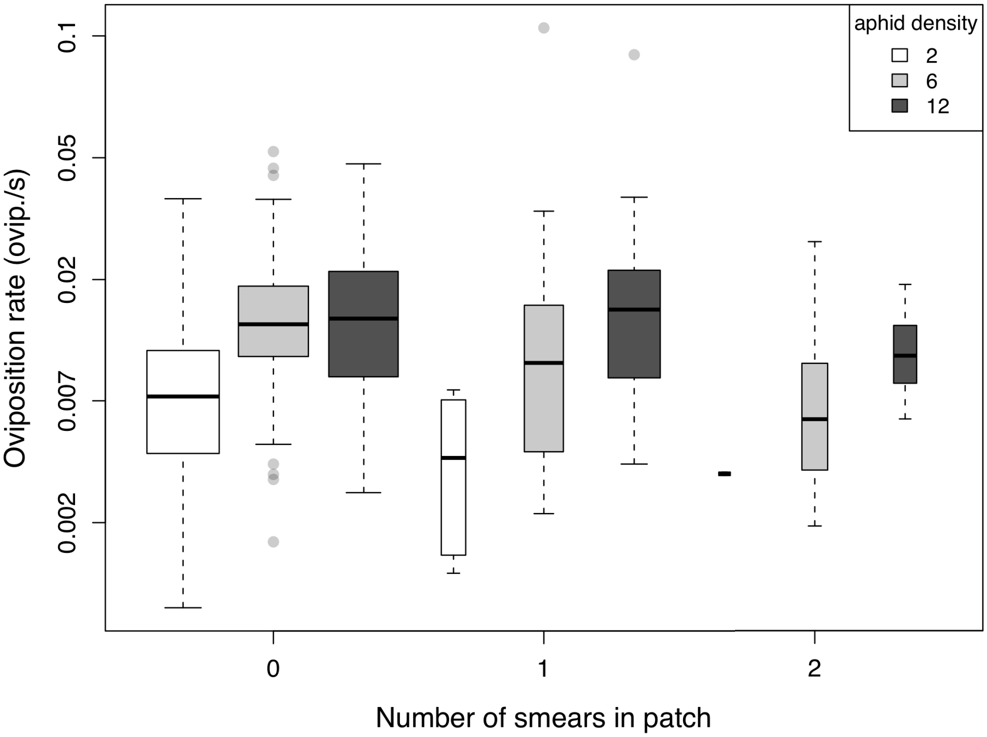

Figure 4.4.2 Parasitoid oviposition rate against smearing frequency in patches. Box plots show the distribution of oviposition rates for patches containing 2 (white), 6 (grey), and 12 (black) aphids. Boxes show the interquartile range (50% of observations), in which the horizontal bar is the median. Whiskers extend 1.5 times the interquartile range beyond the median. Dots show individual observations lying outside this interval. Box widths are proportional to the square root of sample sizes.

4.4.2 Indirect fitness benefits of smearing

Oviposition rate within patches (Figure 4.4.2) decreased significantly with increasing smearing frequency (Wald = 13.1, df = 1, p = 0.0003), and increased significantly with aphid density (Wald = 42.3, df = 1, $p < 0.0001$). Encounter rate with aphids (Figure 4.4.3a) decreased significantly with increasing smearing frequency (Wald = 11.5, df = 1, p = 0.0007), but increased significantly with aphid density (Wald = 66.7, df = 1, $p < 0.0001$). The proportion of encounters resulting in oviposition (Figure 4.4.3b) did not vary significantly with smearing frequency (Wald = 0.03, df = 1, p = 0.87) or aphid density (Wald = 1.84, df = 1, p = 0.17). Similarly, handling time of ovipositions in a patch (Figure 4.4.3c) did not vary significantly with smearing frequency (Wald = 0.27, df = 1, p = 0.60) or aphid density (Wald = 0.16, df = 1, p = 0.69).

The time budget of the parasitoids consisted mostly of foraging and grooming, while little time was generally spent stationary (Figure 4.4.4). The proportion of time spent foraging in the patch (Figure 4.4.4a) decreased significantly with smearing frequency (Wald = 5.0, df = 1, p = 0.025), but not with aphid density (Wald = 0.90, df = 1, p = 0.342). In contrast, the proportion of time spent grooming (Figure 4.4.4b) increased with smearing frequency (Wald = 50.5, df = 1, $p < 0.0001$), but was also unaffected by aphid density (Wald = 2.18, df = 1, p = 0.14). The proportion of time spent stationary (Figure 4.4.4c) was not affected by smearing frequency (Wald = 2.15, df = 1, p = 0.14) or aphid density (Wald = 0.01, df = 1, p = 0.93).

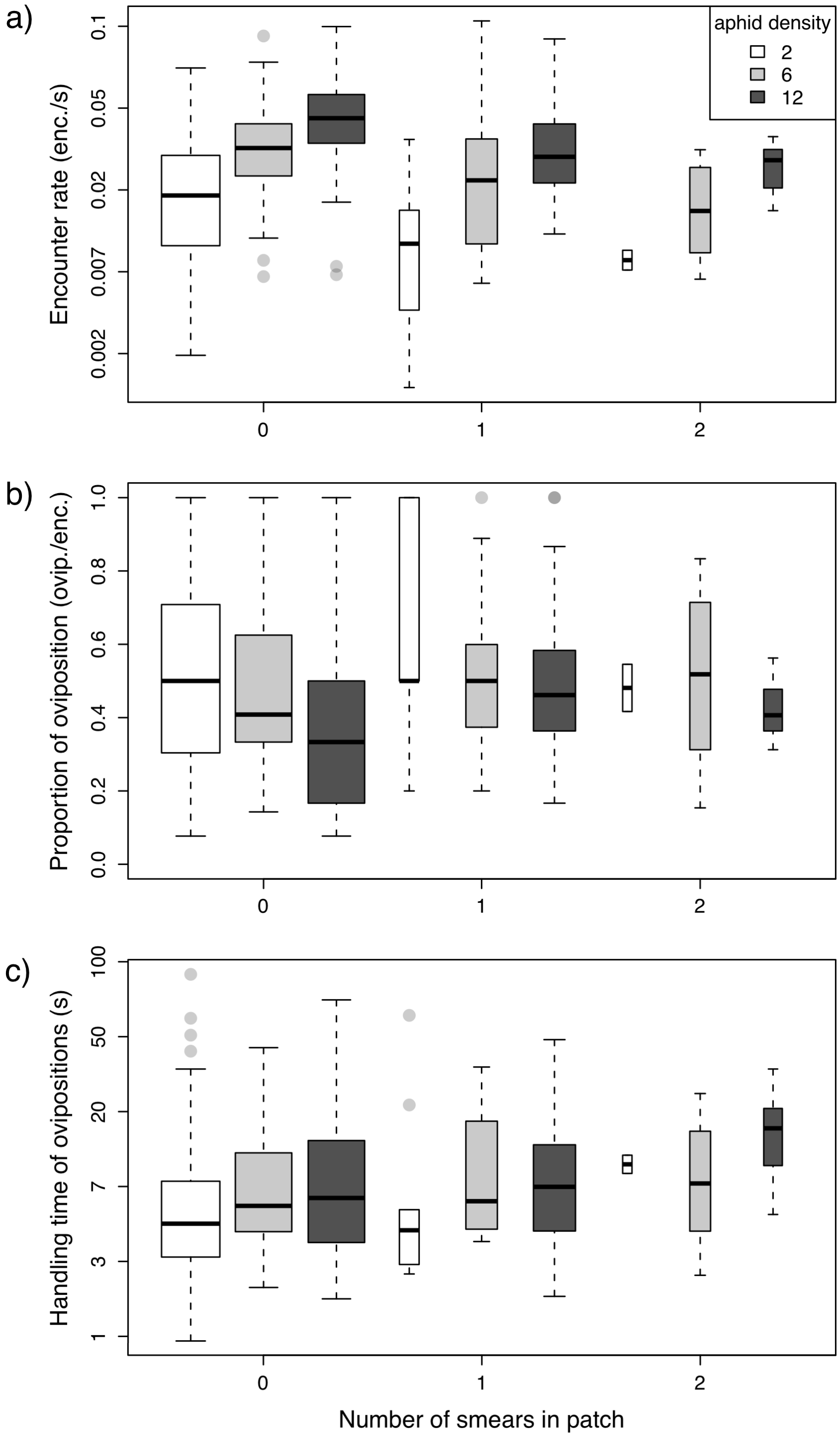

Figure 4.4.3 Components of the parasitoids' foraging success within patch visits against smearing frequency. Box plots show the distributions of: a) encounter rate; b) outcome of encounters; and c) handling time of ovipositions for patches containing 2 (white), 6 (grey), and 12 (black) aphids. Encounter rate (a) and handling time (c) are plotted on a log scale.

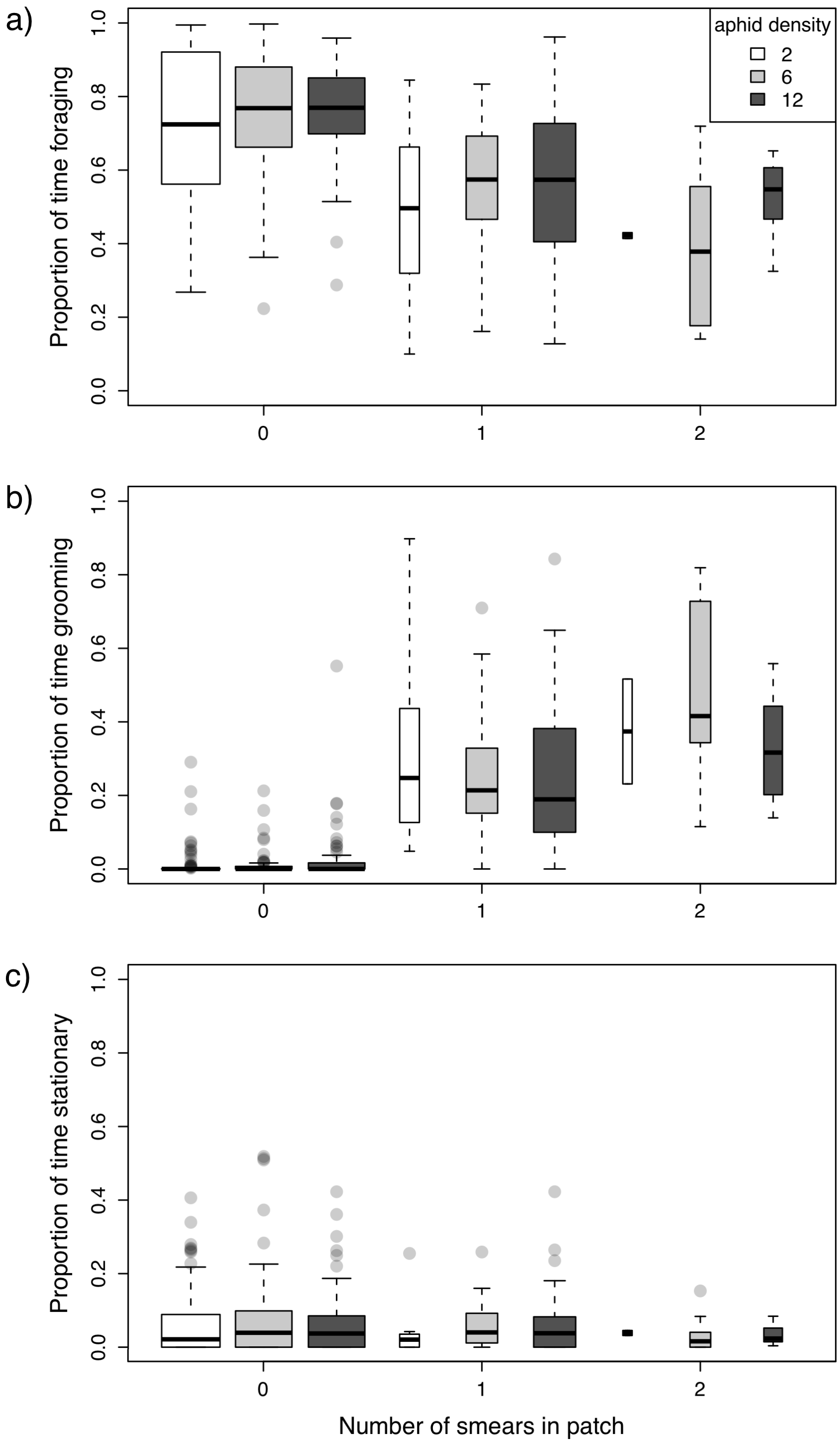

Figure 4.4.4 Parasitoid's time budget within patch visits against smearing frequency. Box plots show the proportion of time spent: a) foraging (searching & handling); b) grooming; and c) stationary in patches containing 2 (white), 6 (grey), and 12 (black) aphids.

4.4.3 Effect of the number of clone-mates on the occurrence of smearing

The proportion of patches in which smearing was observed (Figure 4.4.5) increased significantly with aphid density (Wald=5.49, df=1, p=0.019). In one patch visit lasting only 7 s (aphid density = 6), the parasitoid oviposited in an aphid, was smeared by an aphid, and immediately left the patch. This resulted in a very high smearing rate, but excluding this patch visit did not affect the relationship between the proportion of patches with smearing and aphid density (Wald = 5.60, df = 1, p = 0.018). The proportion of patches with smearing did not increase with the number of encounters in the patch (Wald=0.02, df=1, p=0.88).

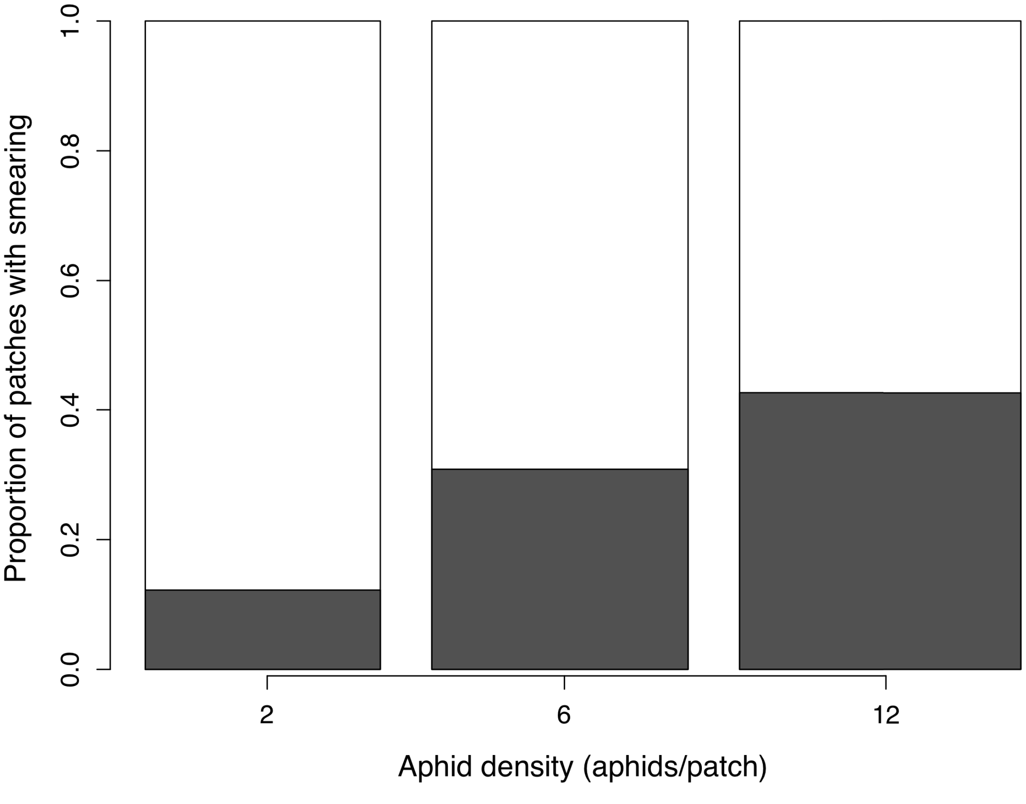

Figure 4.4.5 Occurrence of smearing in patch visits against aphid density. Dark area shows the proportion of patches in which the parasitoid was smeared. The widths of columns are proportional to the square root of sample sizes.

4.5 Discussion

The act of smearing parasitoids with cornicle secretions can be considered altruistic, because it does not reduce the actor's probability of being parasitized, but reduces the parasitoid's rate of oviposition in the colony kin. Moreover, the occurrence of smearing increased with the number of clone-mates in the colony, which is consistent with kin-directed altruism. We discuss these components of altruism in turn.

4.5.1 Absence of direct fitness benefits

For *S. avenae*, there is no direct fitness benefit from smearing the parasitoid *A. rhopalosiphi*, as our detailed analysis found no reduction in the probability of parasitism associated with smearing. On the contrary, aphids that smeared parasitoids were more likely to be parasitized than others, when smearing occurred late in the exploitation of a patch. The absence of direct fitness benefits may be due to smearing occurring once it is too late to prevent parasitism. The specific oviposition behaviour of the parasitoid may contribute to the lack of any direct fitness benefit of smearing. Like many parasitoids of the subfamily Aphidiinae, *A. rhopalosiphi* can sting an aphid and deposit its egg in less than a second (Völkl and Mackauer 2000). The duration of the entire attack sequence, from the encounter to the end of oviposition, was very short (median = 4.4 s) and left little time for the aphid to disrupt its attacker and prevent parasitism.

4.5.2 Evidence of indirect fitness benefits

A parasitoid that has been smeared by a host incurs a reduced oviposition rate within a patch, because it spent less time foraging and more time grooming to remove the hardened cornicle secretions from its body. This, in addition to the effect of the alarm pheromone (which causes neighbouring aphids to express defence behaviours such as kicking, walking away and dropping from the leaf), reduces the parasitoid's foraging efficiency (Barrette et al. 2009). The combined alarm and smearing functions of cornicle secretions provided a considerable benefit for other aphids in the colony, because alerted aphids run away and become unavailable to the parasitoid while it was busy grooming. Consequently, even small increases in grooming time or small reductions in oviposition rate can be costly in terms of lost opportunity cost (Stephens and Krebs 1986). Further studies could aim at quantifying the specific contribution of alarm and smearing mechanism to the reduction of parasitism rate in aphid colonies, and determine whether these effects are additive or synergistic.

Smearing has also been shown to benefit an entire colony of *S. avenae* by reducing the patch residence time of *A. rhopalosiphi* (Outreman et al. 2005). Our study is complementary in showing a benefit to the aphid colony while the parasitoid remains within the patch. This decrease in foraging rate within the patch may be responsible for the shorter patch residence time reported by Outreman and co-workers (2005) if the parasitoid was foraging optimally (Charnov 1976b; Wajnberg 2006). An explicit test of this prediction, however, should consider a possible change in the shape of the parasitoid's fitness gain curve (Giraldeau 2008) that could result from the long periods of grooming following smearing events.

Increasing the number of clone-mates in the colony increases the magnitude of indirect fitness benefits, because a greater number of clone-mates can benefit from smearing. As expected, smearing occurred more frequently when a greater number of clone-mates were present. This result was not simply due to a greater number of encounters or a longer patch residence time in colonies containing more individuals, because our analyses controlled for both. A similar increase in altruistic behaviour with increasing number of kin is found in alarm calls of some birds and mammals (Caro 2005; Wheeler 2008). Further evidence for the altruistic nature of cornicle secretions could be obtained by varying the relatedness of the recipients as is usually done in other systems (West et al. 2006). In colonies of parthenogenetically reproducing aphids, however, individuals are usually clone-mates, so that the number of individuals may be more important than relatedness. Aphids may therefore not have evolved the ability to discriminate kin from conspecifics, as suggested in a recent study of pheromone production in the absence of predators (Verheggen et al. 2009).

4.5.3 Evolution of cornicle secretions

Our results are consistent with the hypothesis that the use of cornicle secretions by *S. avenae* against *A. rhopalosiphi* is altruistic and is maintained through kin selection. Cornicle secretions, however, may not have evolved specifically against aphidiine parasitoids, as aphids are preyed upon by larvae of syrphid flies, predatory midges, coccinellids and other invertebrates, and are also hosts for aphelinid wasps. For most aphidiine parasitoids, attacks and ovipositions may be quick enough to preclude any direct fitness benefits to the aphid releasing cornicle secretions. Aphelinid wasps take longer to oviposit in aphids (Lester and Holtzer 2002), but only trigger the release of cornicle after the end of oviposition. Predators that need to grasp and kill their victims, however, may be more exposed to smearing. For instance, pea aphids (*Acyrthosiphon pisum*) that defend against coccinellid predators using cornicle secretions obtain both a direct (Dixon 1958) and an indirect fitness benefit (Mondor and Roitberg 2004). For pea aphids, cornicle secretions could be better described as mutually benefiting rather than altruistic. Similarly, alarm calls in some birds and mammals may be selfish, benefit a group, or be kin selected, depending on the social context and the type of predator (Caro 2005; Wheeler 2008). In addition, cornicle secretions may be maintained by altruism, but have evolved for selfish benefits. In rodents for instance, the evolution of alarm calls was likely selfish despite evidence of altruism in some species (Shelley and Blumstein 2005). Tracing back the evolutionary history of cornicle secretions in aphids and their associated natural enemies may help determine whether this behaviour initially evolved through selfish or altruistic benefits.

The evolution of altruism can be constrained if the additional offspring of the recipients compete strongly with those of the altruist (West et al. 2002). In fig wasps for instance, brothers compete exclusively among themselves to mate sisters and fight each other to death (West et al. 2001). Increased competition is not a likely constraint in the case of cornicle secretions, because the alarm pheromone also increases the proportion of dispersal morphs in the next generation (Kunert et al. 2005), thereby reducing competition between offspring of the recipient and those of the altruist.

4.6 Conclusions

Our study provides evidence for a case of kin-directed altruistic defence in the grain aphid by showing that cornicle secretions, which are known to be costly, provide no direct fitness benefits to the actor, but instead provide indirect fitness benefits through kin. Moreover, the use of cornicle secretions was consistent with an altruistic behaviour as it increased when the number of clone-mates benefiting from it, and hence the indirect benefit, was greater. This constitutes one of the few examples of kin-directed altruistic defences outside eusocial systems.

PREFACE TO CHAPTER 5

The two previous chapters showed how body size affects the handling time of parasitoids and how cornicle secretions of aphids affect their searching rate. In this chapter, we use results from Chapter 3 to test the hypothesis that host size preference of *A. colemani* is optimal in that it maximizes the value of the host weighted by its handling time.

CHAPTER 5

TESTING COMPETING MEASURES OF PROFITABILITY FOR MOBILE RESOURCES

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(Chapter published in Oecologia)

5.1 Abstract

Optimal diet theory often fails to predict a forager's diet choice when prey are mobile. Because they escape or defend themselves, mobile prey are likely to increase the forager's handling time, thereby decreasing its fitness gain rate. Many animals have been shown to select their prey so as to maximize either their fitness gain or their fitness gain rate. However, no study has yet compared directly these two measures of profitability by generating testable predictions about the choice of the forager. Under laboratory conditions, we compared these two measures of profitability, using the aphid parasitoid *Aphidius colemani* and its host, *Myzus persicae*. Fitness gain was calculated for parasitoids developing in each host instar by measuring life-history traits such as developmental time, sex ratio and fecundity. Fitness gain rate was estimated by dividing fitness gain by handling time, the time required to subdue the host. Fourth instar aphids provided the best fitness gain to parasitoids, whereas second instar aphids were the most profitable in terms of fitness gain rate. Host choice tests showed that *A. colemani* females preferred second instar hosts, suggesting that their decision maximizes fitness gain rate over fitness gain. Our results indicate that fitness gain rate is a reliable predictor of animal's choice for foragers exploiting resources that impose additional time cost due to their mobility.

Keywords Fitness; Handling time; Parasitoids; Optimal diet

5.2 Introduction

5.2.1 Optimal diet theory

Animals are expected to select resources that maximize their lifetime fitness. The optimal diet theory (ODT) has been successful in predicting the diet choice of a large number of animals (Sih and Christensen 2001; Stephens and Krebs 1986). While the ODT works generally well for foragers that exploit motionless or sessile prey, it often fails to predict adequately the choice of foragers when prey are mobile (Sih and Christensen 2001). By escaping or defending themselves, mobile prey add time and energy costs, in the form of either pursuit and capture, both of which reduce the prey's profitability.

Most models estimate prey profitability in terms of fitness return. Direct measures of fitness are difficult to obtain, and biologists usually rely on indirect measures that give absolute values, such as development time, body size, longevity or fecundity (Roitberg et al. 2001). However, in a resource acquisition context, any time cost to the forager must be included in the estimated fitness return associated with a particular resource. When prey defend themselves, their profitability is reduced by the energy and time needed to subdue them. The inclusion of such a behavioural temporal component in the evaluation of a prey's profitability would generate better predictions concerning foragers' choices.

5.2.2 Host choice by parasitoids

The ODT is usually tested in animals that forage for food, but it could also apply to the exploitation of hosts by insect parasitoids. These are ideal model organisms to study the link between host profitability and fitness because the host is the only resource available to the immature parasitoid during its development. Host quality is therefore of critical importance for the fitness of both the immature and adult parasitoids (Brodeur and Boivin 2004; Eggleton and Gaston 1990; Godfray 1994; Godfray and Shimada 1999). Moreover, female parasitoids must also often overcome host behavioural defences. These behavioural defences influence both host profitability and parasitoid behaviour, as in the hyperparasitoid *Syrphophagus aphidivorus* that exploits primary parasitoids present either as a larva in live and mobile aphids or as a pupa in dead and immobile aphids (mummies) (Buitenhuis et al. 2004). Parasitized aphids take longer to be subdued because they are mobile and fight when attacked, whereas aphid mummies cannot escape. As predicted, female *S. aphidovorus* perform better (higher intrinsic rate of increase) and prefer to lay their eggs in mummies rather than in live aphids.

Parasitoids can be confronted with patches of hosts of varying instars and hence quality (Cloutier et al. 2000; Colinet et al. 2005; Harvey et al. 1994). The profitability of parasitoid’s hosts can be measured in terms of the physiological consequences on their offspring. Parasitoid fitness can therefore be estimated from proxies such as body size at emergence, developmental time, longevity and fecundity (Roitberg et al. 2001). It has generally been assumed that late instar hosts, because they provide more nutrients, are more profitable to parasitoids (reviewed by Godfray 1994). However, larger hosts also tend to defend themselves more efficiently than smaller individuals (Brodeur et al. 1996; Walker and Hoy 2003). They can inflict injuries to the parasitoid (Brodeur et al. 1996) and impose time costs that decrease their profitability. An increase in time and energy costs when large prey are consumed has been observed in other species (Griffiths 1980b), such as coccinelids, web spiders or ant-lion larvae, which all take time to subdue large prey that may inflict them injuries. Even sit-and-wait predators, such as constricting snakes, expend a lot of energy and time subduing their prey.

5.2.3 Competing currencies of fitness

When exploiting resources, animals can choose between two strategies: either maximize their gain or maximize their rate of gain (which is often seen as time minimization) (Hixon 1982; Schoener 1971). Which of these strategies should be favoured depends on the constraints the animal is facing. For example, if an animal has fixed energy requirements, it should minimize the time spent acquiring this energy in order to maximize its lifetime fitness. The reverse is expected for animals that have a fixed amount of time to devote to foraging; here, the energy gain should be maximized (Schoener 1971).

Similarly, insect parasitoid females can be either time- or egg-limited (Rosenheim 1999). Female parasitoids can run out of eggs before dying (egg-limited) or, on the other hand, may die without having laid all their eggs (time-limited). Therefore, a maximization of gain rate would suggest that parasitoid females are time-limited while a maximization of the gain would suggest that they are egg-limited.

Although both gain and gain rate have been found to predict the behaviour of different animal species adequately (Cowie 1977; Lemon 1991; van Gils et al. 2003), no single study has compared these two measures of profitability by evaluating their effectiveness at predicting the diet choice of a forager. In this study, we tested whether fitness gain or fitness gain rate best accounted for host selection of parasitoid females.

5.3 Methods

5.3.1 Study organisms

Aphidius colemani (Viereck) (Hymenoptera, Braconidae, Aphidiinae) is a solitary aphid parasitoid of several Aphididae species, including the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera, Aphididae) (Starý

1975). Parasitoid females oviposit in the haemocoel of the host, and during subsequent larval development the immature parasitoid feeds on aphid tissues. At the end of parasitoid larval development, the host is killed, and only its cuticle remains. This so-called ‘‘aphid mummy’’ serves as a shelter, within which the parasitoid spins a cocoon, pupates and emerges as a free-living adult. Parasitoid females have a potential fecundity in the order of hundreds of eggs and are synovigenic (Torres et al. 2007), so they are likely time-limited rather than egg-limited. typically exploit aphid colonies composed of individuals of different instars (Cloutier et al. 2000; Colinet et al. 2005; Kouamé and Mackauer 1991).

Aphids are small, sedentary, plant-sucking insects that often form dense aggregations. *Myzus persicae* has a complex life cycle, involving both sexual and parthenogenetic reproduction as well as seasonal heteroecy. Aphids are exploited by a wide array of natural enemies (predator, parasitoid, entomopathogen) and have evolved a variety of individual (kicking, dropping) and group defences (release of alarm pheromone, dilution effect) (Villagra et al. 2002 and references therein).

A colony of *M. persicae*, established from individuals collected in greenhouses from the Horticultural Research and Development Center (HRDC; Saint-Jean-sur-Richelieu, Canada), was maintained on sweet pepper plants (*Capsicum annuum* L.) *A. colemani* was purchased from Koppert Canada (Scarborough, ON) and reared on *M. persicae*. Insect cultures were maintained at 20 ± 0.5°C and 60–65% relative humidity and under a 16/9-h (light/dark) photoperiod; these were the standard conditions for all experiments.

In order to obtain synchronous *M. persicae* cohorts of a specific age class, about 150 apterous, parthenogenetic adult aphids were placed on a sweet pepper leaf. After 8 h, the adult aphids were removed, and the offspring were reared on excised leaves placed in a Petri dish. Based on the larval developmental time of *M. persicae* under our rearing conditions, first, second, third and fourth instar larvae (L1, L2, L3 and L4) and adult aphids were respectively aged 1, 3, 5, 7 and 9 days.

Parasitoid females used in this study were standardized as follows. Third instar aphids were exposed to female parasitoids for 4 h, at a parasitoid:host ratio of 1:10. Parasitized aphids were then reared in Petri dishes on excised leaves of sweet pepper. Following emergence, males and females remained caged together to allow mating and had access to a dilute honey solution (20%). Prior to each test, 1- to 2-day-old naive females (no previous encounter with hosts) were selected at random.

5.3.2 Life-history trait measurements

A first set of experiments was conducted to measure developmental time, sex ratio and potential fecundity of parasitoid offspring developing in five different aphid instars. Female parasitoids were individually introduced in Petri dishes (diameter 9 cm) containing a sweet pepper leaf with 25 aphids of either L1, L2, L3, L4 or adults. After 4 h, the female was removed and the aphids reared on the leaf until mummification. Aphids were supplied with a fresh excised leaf every 3 days. Mummies were isolated in capsules (300μl Beem polyethylene capsules), and parasitoid emergence was monitored twice daily. Adults were collected and sexed, and females were frozen to prevent further egg production.

The secondary sex ratio (percentage of females at emergence) and development time (oviposition to emergence) were noted. Potential fecundity was estimated by counting the number of mature eggs from the ovaries at the time of emergence. Females of *A. colemani* live for about 5 days when they have access to water and hosts (Hofsvang and Hågvar 1975a), and they can lay up to 88% of their eggs during their first 2 days of life (Hofsvang and Hågvar 1975b). Females were dissected in 1% saline solution within 48 h of emergence, the ovaries extracted from the abdomen under a stereomicroscope, the eggs expelled and the number of mature eggs recorded (a mature egg has a lemon shape).

Twelve experimental blocks were carried out per treatment (host instar), with five females per block each parasitizing 25 aphids of a given instar (L1, L2, L3, L4 and adult aphids). Sixty 60 replicates per instar were carried out (12 blocks × 5 females). In total, 1500 aphids were parasitized (300 per instar). For each life-history trait, the means per instar were calculated for each block.

5.3.3 Handling time

In a second set of experiments, we measured handling time: the duration of a parasitoid attack from the first antennal contact with an aphid host to the end of a successful oviposition. Typically, the following sequence of events leads to a successful oviposition: the foraging parasitoid female contacts the host with her antennae, bends her abdomen forward in preparation of oviposition and then quickly inserts her ovipositor into the host. The tests started by introducing a parasitoid female in an arena (2 × 1.5 × 0.5 cm) with three aphids of a given instar previously placed on a sweet pepper leaf disk (diameter 1 cm). The behaviour of *A. colemani* females was video-recorded for subsequent description and quantification using the software Observer VideoPro version 5.0 (Noldus Information Technology, Leesburg, VA). Hosts were free to walk in the arena and to defend themselves. Each contact with a host leading to an insertion of the ovipositor was considered in the measurement of handling time. Tests ended when 5 min had elapsed without parasitism, and they were discarded if no oviposition occurred in the first 5 min. Females were used only once, and twenty replicates were carried out for each of the five host instars.

5.3.4 Host profitability

To estimate the profitability of each host instar, we used four fitness proxies: potential fecundity, number of female progeny, development time and handling time. For each aphid instar, we estimated two measures of host profitability (currencies): fitness gain and fitness gain rate. The fitness gain was estimated as:

$$\frac{(potential\,fecundity \times number\,of\,females\,produced)}{development\,time}$$

The fitness gain rate was estimated by dividing the fitness gain by the handling time:

$$\frac{(potential\,fecundity \times number\,of\,females\,produced)\ /\ development\,time}{handling\,time}$$

5.3.5 Choice of host instar

We used the ranking derived from the two measures of profitability to test whether *A. colemani* female host selection maximizes fitness gain or the fitness gain rate per host. Paired choice tests were performed to determine the choice of females when offered both L2 and L3 or L3 and adults. These conditions were chosen because they enabled us to distinguish between the two following predictions, assuming that the abundances are sufficient to allow for specialisation. If female choice maximizes fitness gain, they should specialize on L3 over L2 and on adults over L3. On the other hand, if female choice maximizes fitness gain rate, we should observe the opposite choice patterns.

Host instar selection of *A. colemani* females was assessed using paired-choice tests. The order of tests was balanced. Twenty replicates were carried out for each experimental condition. In each test, a female was placed in a Petri dish (diameter 5.5 cm) with 20 aphids (ten of each instar tested). During a test, the female contacted a majority (at least 75%) of the hosts present. The female's behaviour was recorded using the

Noldus Observer XT version 6.0 software. The following behaviours and parameters were either directly observed or derived from the observations.

Observed behaviours:

(1) antennal contact: the parasitoid contacts the aphid with antennae

(2) bending: the parasitoid bends its abdomen

(3) insertion: the parasitoid inserts its ovipositor in the host.

Derived parameter:

(4) Proportion of acceptance: number of insertions / number of antennal contacts.

5.3.6 Statistical analyses

The results were analysed using regression analyses, following verification that the residuals of the regressions conformed to a normal distribution. The best model was selected based on the Aikake Information Criterion (AIC), a measure of the goodness of fit of an estimated statistical model (Akaike 1974). For life-history trait measurements, the sample sizes (>40 per model tested) were large enough to use the AIC values directly (Anderson and Burnham 2002) while for handling time, we used the AICc (AIC corrected for small sample size; Anderson and Burnham 2002). The extent of specialization was established by testing whether the frequencies of antennal contact, abdomen bending and ovipositor insertion of the ovipositor between host instars were significantly different from a 50:50 ratio based on chi-squared analysis.

5.4 Results

5.4.1 Life-history traits measurements

Development time of *A. colemani* decreased with increasing host instar. Parasitoids took almost 20% longer to develop when eggs were laid in L1 hosts than when laid in adult hosts. For any given host instar, parasitoid males developed faster than females (Figure 5.4.1a).

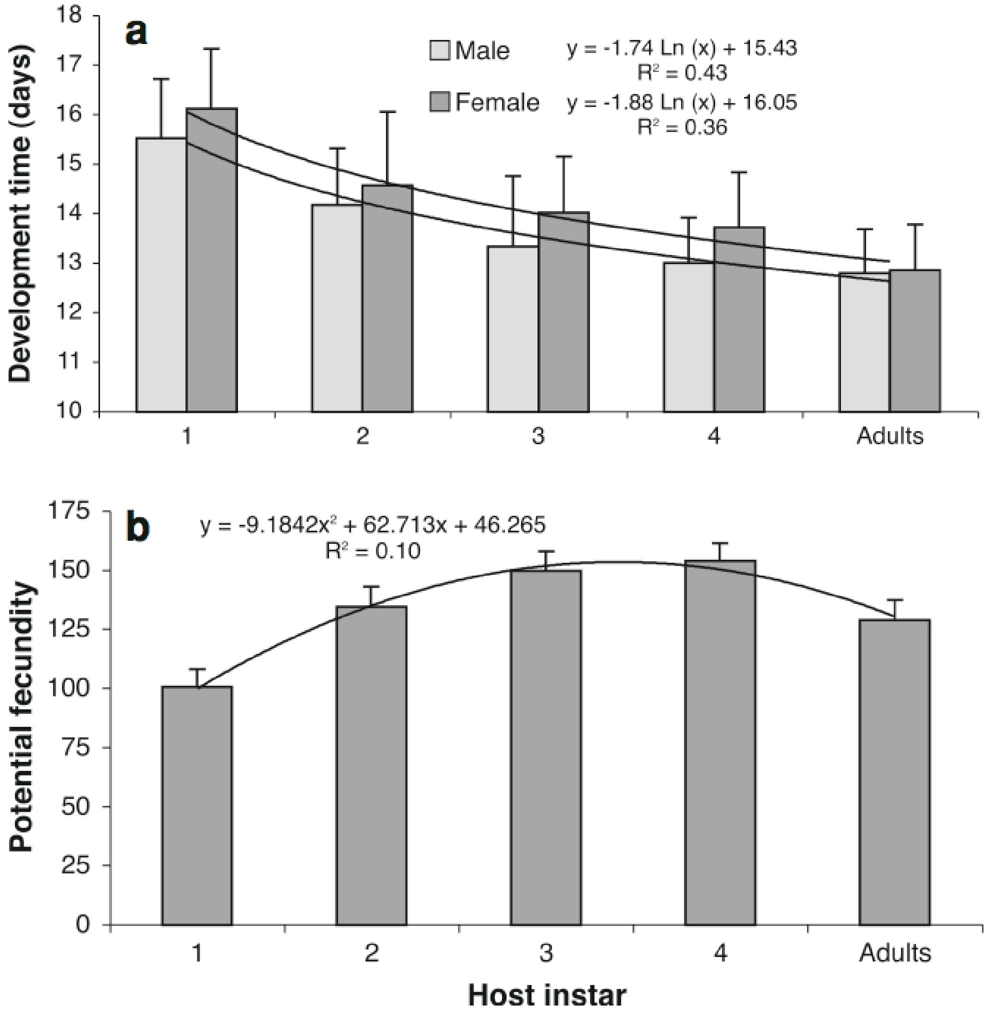

Figure 5.4.1 Influence of host instar on development time (a) and fecundity (b) of *Aphidius colemani* parasitizing *Myzus persicae.*

Potential fecundity at emergence increased with host instar and was maximum when females developed in L4 aphids (Figure 5.4.1b).

5.4.2 Handling time

Handling time increased with increasing host instar (Figure 5.4.2), and females took more time to parasitize older hosts than younger hosts. Time to parasitize an adult aphid was fourfold longer than that for an L1 aphid.

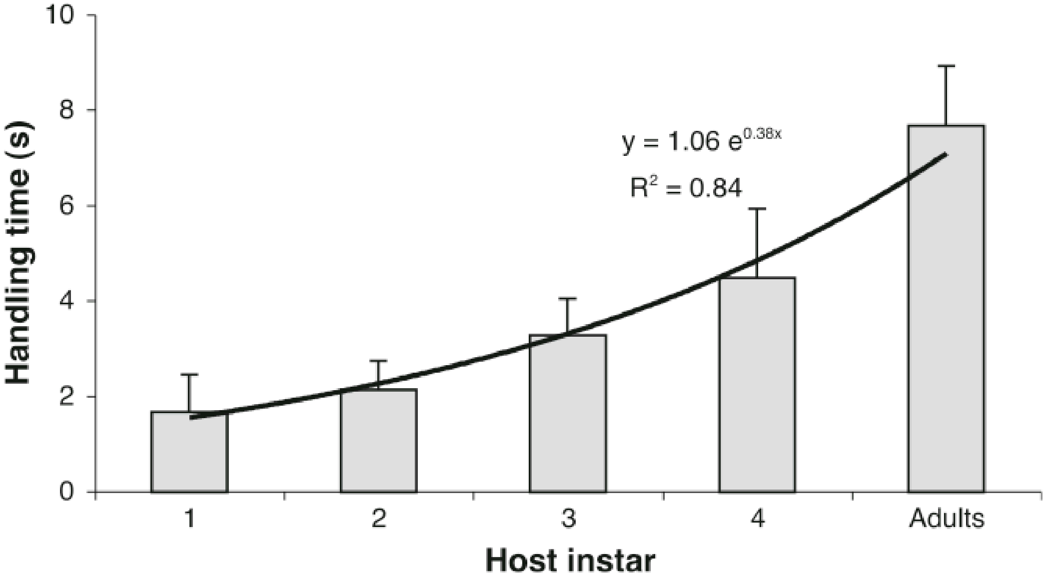

Figure 5.4.2 Influence of host instar on the handling time of *A. colemani* parasitizing *M. persicae.*

5.4.3 Measures of profitability

Fitness gain increased with host instar, with the L4 aphids representing the best fitness return per host for the parasitoid and the L1 aphids being the least profitable (Figure 5.4.3a). However, when handling time was integrated into the measure of profitability, the relative overall profitability of aphid instar changed, and fitness gain rate was maximized for L2 hosts and declined with older host instars (Figure 5.4.3b).

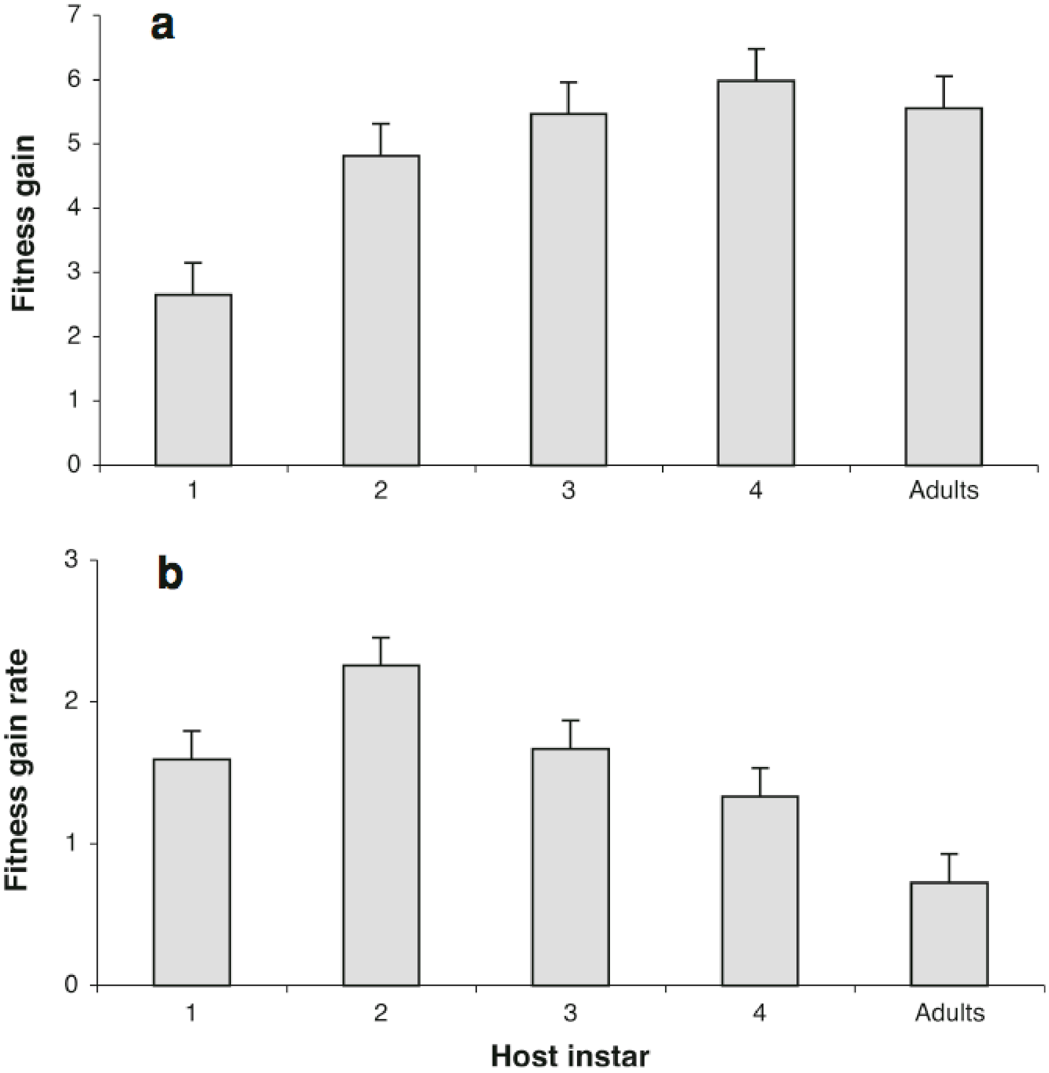

Figure 5.4.3 Host profitability as a function of host instar in *A. colemani* parasitizing *M. persicae*. Fitness gain per host estimated with life-history traits measurement (a); and fitness gain rate estimated with life-history traits measurement and handling time (b).

5.4.4 Choice of host instar

Based on host acceptance, parasitoid females significantly preferred L2 over L3 aphids and L3 aphids over adult aphids (Table 5.4.1). Of significance, for the L2 versus L3 host pair, females expressed their preference for L2 hosts even though they made more contacts with L3 hosts; i.e. they actively rejected the latter more frequently (Table 5.4.1). Frequencies of bending and oviposition behaviours followed the same pattern; females bent their abdomen and inserted their ovipositor more often with L2 aphids than with L3 and more often with L3 than adult hosts.

Table 5.4.1 Number of contacts, abdomen bendings and ovipositions of *Aphidius colemani* parasitizing different instars of *Myzus persicae* in the choice tests.

Choice	Host instar[a]	*n*	n_{contacts} (mean ± SE)	n_{bending} (mean ± SE)	$n_{\text{ovipositions}}$ (mean ± SE)	Proportion of acceptance (mean ± SE)[b]
L2 versus L3	L2	15	11.87 ± 0.87b	9.13 ± 0.80a	9.00 ± 0.80a	0.75 ± 0.04a
	L3		16.67 ± 2.05a	6.27 ± 0.95b	3.47 ± 0.72b	0.19 ± 0.03b
L3 versus adults	L3	15	13.13 ± 1.29a	10.00 ± 0.88a	9.80 ± 0.86a	0.76 ± 0.03a
	Adults		15.13 ± 1.62a	4.07 ± 0.73b	1.47 ± 0.27b	0.09 ± 0.02b

Within an experimental condition, values followed by a different letter denote a significant difference between host instars (Chi test $\alpha = 0.05$).

[a] L2, L3, Second and third instar larvae, respectively

[b] The proportion of acceptance represents the number of ovipositions in a given instar divided by the number of antennal contacts. SE, Standard error.

5.5 Discussion

Our results highlight the importance of taking time into account when estimating the profitability of diet items used to predict an animal's choice. In the case of parasitoids exploiting hosts that can defend themselves, fitness gain rate was a better currency predicting animal's choice than simply fitness gain. The result suggests that parasitoid females assess host profitability from a combination of physiological characteristics and handling time.

Although many studies have shown that animals maximize their rate of gain (Stephens and Krebs 1986), surprisingly few studies have tested concurrently two currencies to determine which is the best predictor of behavioural decisions (and see Kacelnik 1984; Ydenberg et al. 1994 for discussion). One example of such a test involves a study on central place foraging in starlings (*Sturnus vulgaris*), which confronts different predictions derived from four optimality models (Kacelnik 1984). Kacelnik (1984) found that maximization of the energy available for growth (a measure called ‘‘family gain’’ in his paper) was the best predictor of parent foraging decisions. To our knowledge, our study is the first that tests alternative fitness currencies in an arthropod and which shows that fitness gain rate is a better predictor of behavioural decisions than fitness gain.

5.5.1 Fitness gain measures: development time and fecundity

Our results support the expected concordance between fitness gain and common life-history traits in parasitoids. For instance, development time of *A. colemani* decreased with increasing host instar, a consistent pattern for parasitoids that can attack different stages of the same host species (Harvey et al. 1994; Mackauer and Sequeira 1993). Koinobiont parasitoids (whose hosts continue to feed, grow and develop following parasitization) adapt their growth trajectories to the nutritional and physiological attributes of the host. Likely, *A. colemani* developing in early aphid instars would favour increased body size and fecundity (the two traits being correlated) over a shorter development time. However, those developing in large aphid nymphs or adults would allocate extra nutritional resources to maximize all life-history traits, as shown by their potential fecundity (Figure 5.4.1b). Differential development rates may have important consequences on both parasitoid survival and competitive interactions. A shorter development time would reduce the risks of mortality from natural enemies, as predicted by the slow-growth-high-mortality hypothesis

(Benrey and Denno 1997). Parasitoids that develop faster would also have the competitive advantage of being the first ones to exploit resources from the habitat.

Reproductive success in *A. colemani* is correlated with host size at the time of parasitization with L4 hosts providing females with the highest potential fecundity. Similar results have been reported for *Aphidius nigripes* (Cloutier et al. 2000), but our results do differ from those of (Qayyum 2001), where the potential fecundity of *A. colemani* reared on *M. persicae* increased linearly with host age. However, in the latter study, a different biotype of parasitoid was used, and the aphids were reared on a different plant species (*Brassica oleracea*), which possibly modified the impact of host instar on the females' fecundity.

Aphidius colemani does not perform equally well on all instars of *M. persicae*. Based on our measures of life-history traits, parasitoid females should select L4 instar aphids if they rely on fitness gain to estimate host profitability.

5.5.2 Fitness gain rate measures: handling time

Handling time generally increases with prey size (Griffiths 1980b), as has been observed in shore crabs (*Carcinus maenas*) where handing time has recently been demonstrated to be the most important factor explaining their prey-handling behaviour (Rovero et al. 2000). Similar results can also be found in other animals [insects, (Griffiths 1980a); fish, (Gill and Hart 1994); mammals, (Griffiths 1980b); a number of aphid parasitoid species: *Ephedrus cerasicola*, (Hofsvang and Hågvar 1986); *Monoctonus paulensis*, (Chau and Mackauer 2001a); *Lipolexis oregmae*, (Walker and Hoy 2003)].

In our study, late host instars were more profitable to *A. colemani* in terms of fitness gain, but they imposed a significant cost because of increased handling time. Handling time, which included time required to subdue host and oviposit, increased with increasing host instar, an expected trend as older aphids tend to escape and fight more frequently and more aggressively than younger aphids (see Villagra et al. 2002 and references therein). Parasitoid females thus have to invest more time to parasitize an older host. *Aphidus colemani* does not have the same ability to attack all instars of *M. persicae*. Based on our estimation of handling time, parasitoid females should neglect large hosts if they rely on fitness gain rate to estimate host profitability.

5.5.3 Profitability and choice of host instar

The ODT predicts that an organism should specialize on the most profitable resource when sufficiently abundant and become a generalist with no preference otherwise. Our findings do not support either prediction; parasitoid females showed behavioural plasticity towards host acceptance but expressed partial preferences for one host instar. This preference for the host that provided the highest rate of gain (as seen in host choice test) is consistent with the hypothesis that the parasitoids have been selected to maximize gain rate rather than simply gain. The type of partial preference we have observed for *A. colemani* has been reported in virtually every test of ODT (Stephens and Krebs 1986) and is usually attributable to violations of some of the model’s assumptions. For instance, it is unlikely our female parasitoids were omniscient. More likely, they had to sample an aphid patch to learn how many hosts of each type were available. Moreover, females did not experience unchanging rates of encounter with each host type within the patch as they parasitized the preferred hosts. As patch quality declined with the abundance of the most profitable hosts, the parasitoid female reduced the density of the most profitable host such that a generalist policy became optimal. We are confident, therefore, that the host preferences we observed in our tests indicate that parasitoids have been selected to maximize gain rate rather than total gain.

Maximizing the rate of fitness gain rather than fitness gain should be optimal in other activities, such as reproduction, where animals look for resources and where costs are important. Male insect parasitoids can be limited in either their sperm stock or time (Boivin et al. 2005; Damiens and Boivin 2006). The fact that males adjust their patch residence time based on the availability of virgin females tends to support the fact that males are selected to optimize the rate of female acquisition rather than the total number of females mated per patch (Martel et al. 2008).

This maximization of gain rate suggests that *A. colemani* females are time-limited. Time limitation in parasitoids is expected when the female is likely to die before having laid all its eggs (Rosenheim 1999; Sevenster et al. 1998). Our data are consistent with the notion that parasitoid females can assess their rate of fitness gain, but we have not identified the proximate mechanisms involved. Several hypotheses could be examined. Recently, the egg parasitoid *Trichogramma brassicae*, has been shown to have a fixed innate estimate of habitat quality and to exploit host patches according to this estimate (Wajnberg et al. 2000). Such innate estimates would be expected for specialist species, but they are unlikely to be effective for generalist parasitoids, such as *A. colemani*, which can parasitize hosts from different species that vary in terms of size and quality (18 species of aphids in Southeastern Europe; Kavallieratos et al. 2004). For such generalist species, estimation of host profitability is more likely learned. Mechanisms such as memory window could provide an animal with an estimation of the average rate of gain based on its last encounters (Valone 1992) and has already been proposed to explain how parasitoids could forage optimally (Pierre et al. 2003). Alternatively, parasitoid females may base their estimate of host profitability on their first few encounters. Recent empirical evidence of this mechanism has been reported in the egg parasitoid *Anaphes victus* that estimates patch quality based on the first patch encountered rather than on a fixed innate estimate (Boivin et al.

2004). Which proximate mechanisms parasitoid females are using to assess their fitness gain rate remain to be examined.

PREFACE TO CHAPTER 6

The previous chapters showed in a model system that handling time of an aphid parasitoid is affected by body size (Chapter 3) and that host size preferences were dependent on this handling time (Chapter 5). Chapters 6 and 7 attempt to determine whether these results scale up to the level of ecological communities. Phylogenetic methods are used to study phylogenetically related species of parasitoids and hosts. Chapter 6 uses data from the literature to test the hypothesis that handling time is determined by the relative body size of parasitoid and host species.

CHAPTER 6

BODY SIZE OF HOST, BUT NOT PARASITOIDS DETERMINES HANDLING TIME IN APHID-PARASITOID INTERACTIONS

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(Chapter to be submitted to Evolutionary Ecology Research)

6.1 Summary

1. Handling time associated with resource exploitation is usually assumed to be inversely related to the ratio of consumer:resource body sizes (relative body size hypothesis). This relationship has been shown within specific predator-prey and parasitoid-host associations. However, it remains unclear whether this pattern emerges consistently among systems.
2. We tested the hypothesis that handling time decreases with increasing consumer:resource size using a phylogenetic comparative study of 63 behavioural observations from 27 aphid-parasitoid associations. Parasitoid species averages, aphid species averages, and individual species associations were analyzed.
3. Body sizes of aphids and parasitoids were conserved with respect to the phylogeny of aphids, but handling time and parasitoid:aphid body size ratio did not. Analyses of parasitoid averages revealed no significant relationship between handling time and consumer:resource size, parasitoid body size, or aphid body size. Results from aphid averages, however, showed that handling time increases with aphid body size and decreased with parasitoid species body size. For analyses of individual species associations, handling time increased with aphid body size, but did not vary with parasitoid body size, or their ratios.
4. Our comparative study did not support the relative body size hypothesis for aphid-parasitoid associations. Instead, aphid body size was identified as the only consistent predictor of handling time. In addition, the results from parasitoid species data illustrate how conducting comparative studies of foraging traits strictly from the upper trophic level can leave out important information.

Key-words: foraging behaviour, handling time, phylogenetic comparative method, body size ratio, aphid parasitoid

6.2 Introduction

Foraging animals incur time and energy costs when pursuing, subduing, or killing prey or hosts. These costs are usually approximated by handling time, a key parameter in individual behaviour (Sih and Christensen 2001; Stephens and Krebs 1986; Turesson et al. 2002), population dynamics (Brose 2010; Holling 1959), and food web structure (Brose 2010; Petchey et al. 2008). Following the relative body size hypothesis (Griffiths 1980b), handling time is inversely related to the relative predator:prey size. Studies of foraging behaviour at the individual level support this hypothesis within numerous taxa. A few recent examples include copepods (Rao and Kumar 2002), isopods (Aljetlawi et al. 2004), insect predators (Faria et al. 2004), insect parasitoids (Barrette et al. 2009; Henry et al. 2009), fishes (Johansson et al. 2004), lizards (Cooper Jr and Anderson 2006), snakes (Cruz-Neto et al. 2001), birds (Slagsvold and Sonerud 2007), and mammals (Caro 2005). The decrease in handling time with increasing consumer:resource (C:R) size appears to be a general pattern within single C:R associations, and contributes to explain diet choices of many foragers exploiting different sized individuals of a given resource species (Barrette et al. 2009; Chen and Jiang 2006; Hjelm and Persson 2001; Johansson et al. 2004), despite some exceptions (Carlson 1992; Christensen 1996).

More recently, the relative size hypothesis has also been used to predict patterns of species associations in foraging-based food web models (Beckerman et al. 2006; Costa et al. 2008; Petchey et al. 2008). These models assume that handling time, a key parameter in these models (Petchey et al. 2008), decreases with C:R. However, there is little evidence that the relative size hypothesis holds for differences in species body sizes of multiple consumers and resources in the context of food webs. Different behavioural and morphological adaptations of consumers and resources may obscure this relationship. Moreover, the diet of consumers, and consequently their foraging behaviour, may be determined in part by their evolutionary history (Blomberg et al. 2003). Finally, handling time, and other foraging traits involve in predator-prey interactions, are often considered traits of the consumer (Goss-Custard et al. 2006). These traits, however, are likely determined by the resource species as well, so that comparative studies should consider both phylogenies.

We tested the hypothesis that handling time decreases with increasing C:R using a comparative study of aphid-parasitoid associations. Female aphid parasitoids reproduce by laying eggs in their hosts, which can defend themselves and impose a handling time cost to their attackers. Within a given aphid-parasitoid association, parasitoids handle more quickly the younger and smaller instars of their hosts (Chau and Mackauer 2000; Chau and Mackauer 2001a; Hofsvang and Hågvar 1986; Weisser 1994), and smaller parasitoids have longer handling times than larger parasitoids (Henry et al. 2009). The relationship between handling time and parasitoid size, aphid size and C:R were tested using phylogenetic comparative methods. Moreover, analyses were conducted using the average traits for parasitoids and their phylogeny, the average traits for aphids and their phylogeny, and using individual aphid-parasitoid associations and both phylogenies simultaneously.

6.3 Materials and methods

6.3.1 Study system

We focussed our study on aphids (Hemiptera: Aphididae) and one of the two main groups of aphid parasitoids, the Aphidiinae wasps (Hymenoptera: Braconidae). Aphids are small plant sucking insects that feed on a wide range of herbaceous plants, trees and agricultural crops worldwide (Dixon 1977). Populations of aphids go through several generations of parthenogenetic viviparous reproduction, during which they form large multigenerational colonies of clones (Loxdale 2008 and references therein). Newborn aphids increase in size through their four successive nymphal stages (instars) and adult stage. The adult body length of aphid species varies from less than 1 mm to over 5 mm (Blackman and Eastop 2006b). Aphids display a wide range of individual defence behaviours when attacked by predators or parasitoids, including kicking, swivelling, and dropping off their plant. They can also produce sticky cornicle secretions containing alarm pheromones (Gross 1993). Aphidiinae are small wasps that range from about 1 mm to 8 mm in length (Medvedev 1995; Starý 1988). Females reproduce by laying a single egg in the host haemocel. Following hatching and during subsequent instars, the parasitoid larva feeds on host tissues. The last instar kills the aphid host and pupates within the aphid skin (mummy), from which it will emerge as an adult wasp.

6.3.2 Dataset

Handling time is defined here as the duration of a parasitoid-host encounter resulting in stinging by the parasitoid, starting from the first contact of a host with the parasitoids' antennae to the end of the stinging behaviour. Handling time measures obtained from direct behavioural observations were gathered from the literature by using the search expression *((“handling” or “behavio”) and “aphid”)* in biological and agricultural electronic databases (Agricola:1970-2008; AGRIS:1975-2008; BIOSIS:1969-2008; CAB:1973-2008) from WebSPIRS (Ovid Technologies, 2009). Any articles whose abstract mentioned behavioural observations between parasitoids and aphids were checked for handling time measures. The search was extended to references in the selected papers as well as attempts to communicate with their authors. Sixty-three measures of handling time from behavioural observations were found for various instars of aphids in 27 different aphid-parasitoid species associations. The aphid instars used in the studies were noted and ranked from 1-5 (four larval instars and adult). In one instance, the attacked instar was not known and the known preferred instar was used. In two instances, the average instar available to the parasitoid was used. The reported handling time for the parasitoid *Aphidius picipes* (308s) was excluded from our study because the recorded time included an atypical lengthy “wing fluttering” behaviour during which the parasitoid was not in contact with the host (van Baaren et al. 2004).

Total aphid body length (tip of head to abdomen, excluding cauda) was measured from slide-mounted specimens at the Canadian National Collection of Insects (CNCI) (Ottawa, Canada). Digital images of adult apterous aphids (wingless parthenogenetic form) were obtained using a Nikon Coolpix P5000 digital camera mounted on one tube of a Nikon SMZ-U stereoscope (0.75X-7X magnification). Images were calibrated at each magnification using a 100µm graduated slide and linear morphological measurements were taken using ImagePro 5. For parasitoid species, digital images of dried specimens at the CNCI were obtained using the same technique as above. Total body length was obtained by adding linear measurements of the head, thorax, and fitted splines through the curved abdomen of the dried specimens. Specimens of one aphid species and 18 parasitoid species were unavailable at the CNCI, so data from the published literature were used.

Phylogenies of parasitoids and aphids were constructed based on published molecular phylogenies. For parasitoids, a phylogenetic tree (Figure 6.4.1) was constructed from five molecular phylogenies that focused on Aphidiinae (Belshaw et al. 2000; Chen et al. 2002; Kambhampati et al. 2000; Sanchis et al. 2001; Smith et al. 1999). Ambiguous relationships were left as unresolved nodes. For aphids, the phylogenetic tree (Figure 6.4.2) was constructed from two recent studies (Kim and Lee 2008; von Dohlen et al. 2006), which were in agreement with three other published molecular phylogenies for the species included in our study (Normark 2000; Ortiz-Rivas et al. 2004; Sabater et al. 2001). Because the phylogenetic trees were constructed from different phylogenies, arbitrary branch lengths were determined following Grafen (1989), with a branch length exponent (rho) of one.

6.3.3 Statistical analyses

Handling times were first corrected for the different instars used by obtaining the residuals of the regression between handling time and instar rank. The regression was fitted using a generalized estimating equation (Zeger and Liang 1986) to account for differences among aphid-parasitoid associations. Handling time was modelled as a Gamma distribution (log link function), and the working correlation structure was set to "independence" because the true correlation structure was unknown (Pan 2001). The resulting instar-corrected handling times were normally distributed, but aphid and parasitoid body lengths were log-transformed to achieve normality.

For the analyses of parasitoid data and aphid data, variables were first tested for the presence of a phylogenetic signal with their respective phylogenies using the test for serial independence (Abouheif 1999; Pavoine et al. 2008) and the K statistic (Blomberg et al. 2003), which assumes an Ornstein-Uhlenbeck (OU) model of trait evolution with absolute limits or stabilising selection constraining trait variation (Martins et al. 2002). Handling time was then modelled against parasitoid size, aphid size, and C:R using phylogenetic generalized least squares (PGLS) regressions (Paradis 2006). The amount of phylogenetic signal in the relationship was estimated in the PGLS model, assuming an OU model of trait evolution. Finally, the analysis of individual aphid-parasitoid associations was conducted using a phylogenetic bipartite linear model for the analysis of trophic associations (Ives and Godfray 2006), to account for both parasitoid and aphid phylogenetic dependence simultaneously (assuming an OU model of evolution). Phylogenetic analyses were also conducted using a simpler Brownian motion model of trait evolution (Felsenstein 1985) with consistent results (not shown). Statistical analyses were performed in R version 2.9.1 (R Development Core Team 2009), with the package "ape" version 2.3 (Paradis et al. 2004) for phylogenetics. The tests for phylogenetic signals were conducted using the function abouheif.moran() in the package "adephylo" version 1.0.0 (Jombart and Dray 2008) and phylosignal() in the package "picante" version 0.7.0 (Kembel et al. 2009). PGLS were conducted using the function gls() in the package "nlme" (Pinheiro et al. 2009), and the phylogenetic bipartite linear model using a modified version of the function pblm() from the package "picante" version 0.7.0 (Kembel et al. 2009). The latter was modified to include C:R as a covariate unique to each aphid-parasitoid association (code available on request). The threshold of significance was set to 0.05 for all statistical tests.

6.4 Results

Handling times varied in orders of magnitude (2-125 s), while variation in aphid body length (1.4-4.4 mm), parasitoid body length (1.7-3.9 mm), and C:R (0.53-1.5) varied more moderately.

6.4.1 Parasitoid species means

There was no significant phylogenetic signal in handling time or any of the covariates with respect to the phylogeny of parasitoids, using the test for serial independence or the K statistic (all $p > 0.05$). The average handling time of parasitoids did not vary with their body lengths (Wald-t = -1.4769, df=1, p = 0.1655), the average body length of their aphid hosts (Wald-t =

1.5771, df=1, p = 0.1408), or their average C:R (Wald-t = 0.9897, df=1, p = 0.3419).

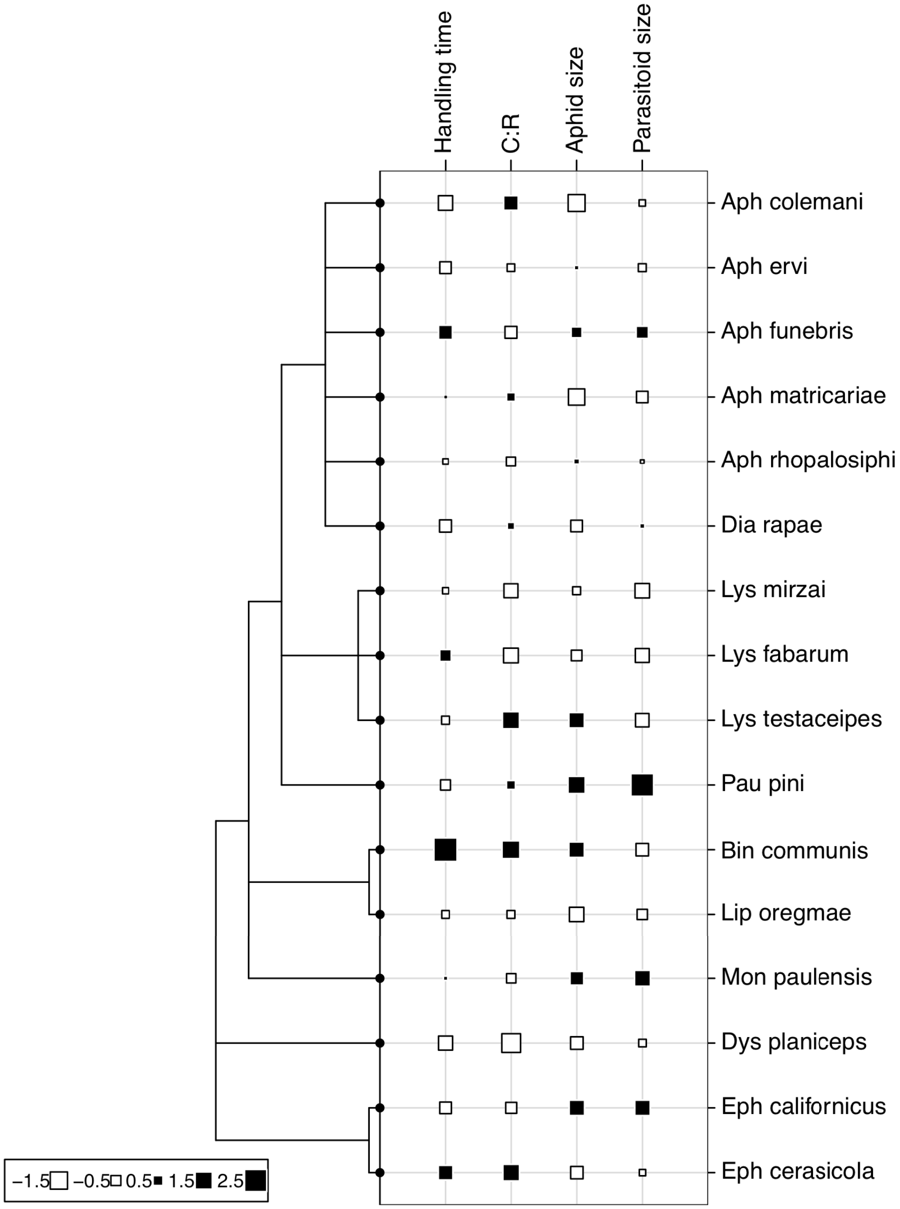

Figure 6.4.1 Trait averages for each parasitoid species plotted on the parasitoid cladogram used. Handling times are corrected for aphid instar; aphid and parasitoid size are log transformed body lengths. Traits were scaled and centred for illustrative purposes. There was no significant phylogenetic signal in any traits.

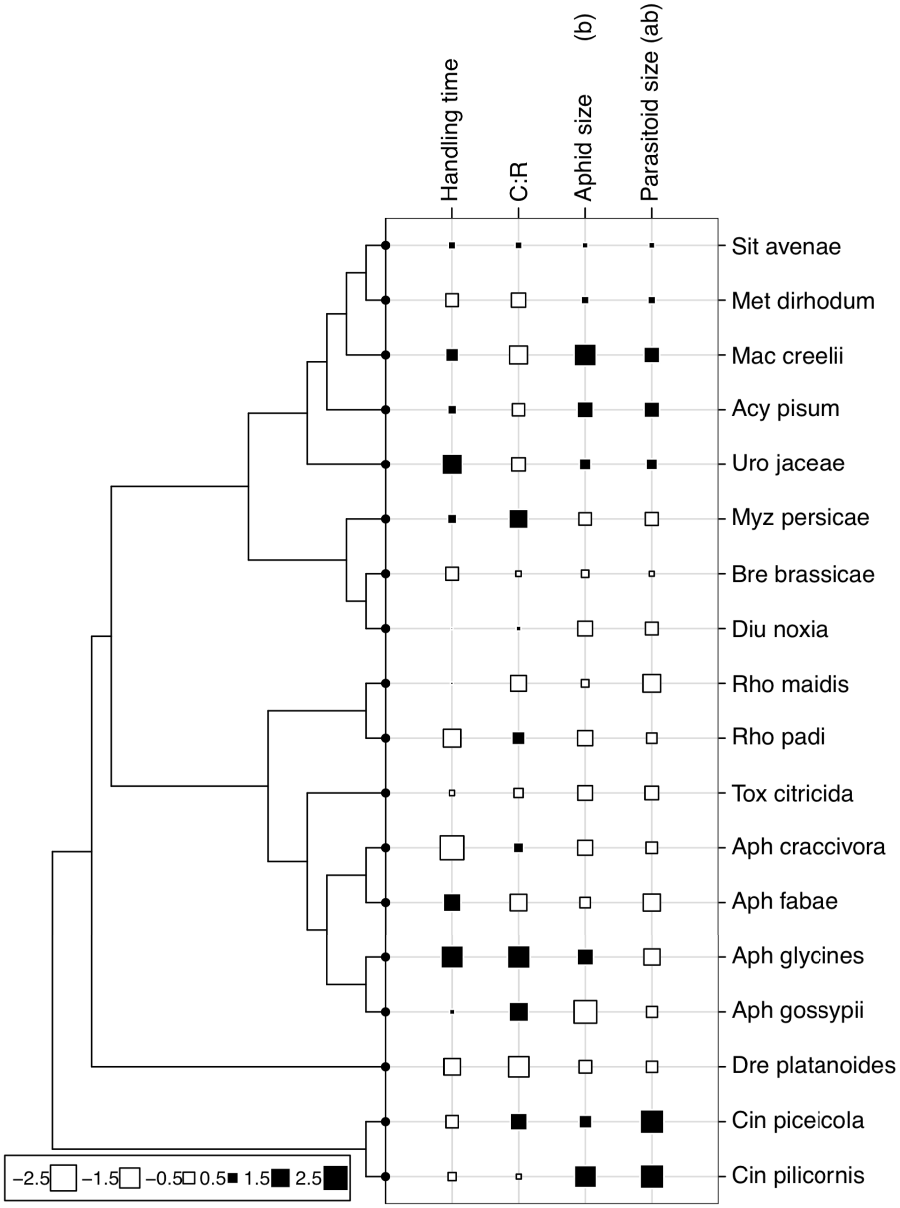

Figure 6.4.2 Trait averages for each aphid species plotted on the aphid cladogram used. Handling times shown are corrected for aphid instar; aphid and parasitoid size are log transformed body lengths. Traits were scaled and centred for illustrative purposes. Letters in brackets denote traits with significant phylogenetic signal using (a) Abouheif's test for serial independence and (b) Blomberg's K statistic.

6.4.2 Aphid species means

There was a highly significant phylogenetic signal in parasitoid size on the phylogeny of aphids using the test for serial independence or the K statistic (p's < 0.001). Aphid size showed a marginally significant signal in aphid phylogeny using the K statistic only (p=0.047). Neither handling time nor C:R showed a significant phylogenetic signal with both methods (all p > 0.05). The average handling times for aphid species increased with aphid species body length (Wald-t = 2.8123, df=1, p = 0.0138), and decreased with mean parasitoid species body length (Wald-t = -2.2057, df=1, p = 0.0446), but did not vary significantly with C:R (Wald-t = 0.8768, df=1, p = 0.3954).

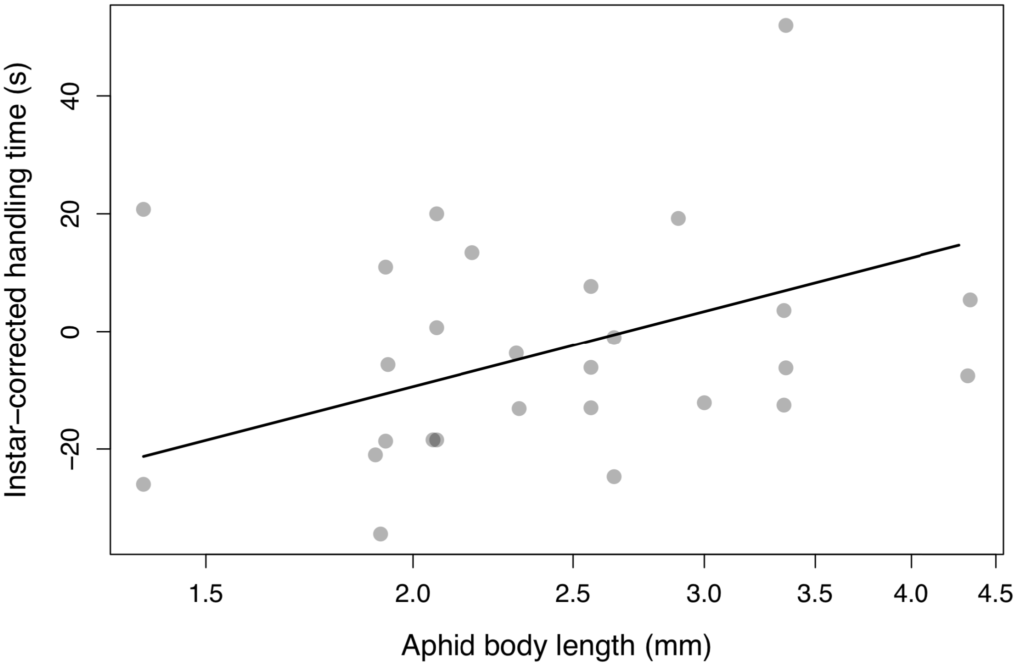

Figure 6.4.3 Aphid instar corrected handling time increased with aphid species body length (log scale) in individual aphid-parasitoid associations.

6.4.3 Individual aphid-parasitoid associations

The handling times in individual aphid-parasitoid associations increased with aphid body length (Z = 2.1639, df=1, p=0.0304; Figure 6.4.3), but did not vary significantly with parasitoid body length (Z = -1.8149, p = 0.0696) or with C:R (Z = -1.2868, p = 0.1982).

6.5 Discussion

Our results do not support the relative size hypothesis for aphid-parasitoid associations, as handling time did not vary significantly with consumer:resource body size ratio (C:R). Handling time increased significantly with aphid body size in analyses of aphid species data and individual aphid-parasitoid associations, and was related to parasitoid size only marginally in the analysis of aphid species data. These results and the absence of association between handling time and any body size measure in the analysis of parasitoid species data are discussed in relation to parasitoid foraging behaviour. More general implications for comparative studies of foraging and other traits that result from interspecific interactions are also discussed.

6.5.1 Body size and handling time in aphid parasitoids

The lack of a clear relationship between handling time and parasitoid body size or C:R among tested aphid-parasitoid associations differs from studies conducted with one species of parasitoid (Henry et al. 2009; Wu et al. 2011). The result is not likely due to a lack of variability the data, because it increased significantly with increasing aphid body size. The range of C:R, however, was more modest. There was no evidence of evolutionary constraint on handling time, because like most behaviours

(Blomberg et al. 2003), handling time showed no phylogenetic signal. Specific adaptations of parasitoids may explain differences in handling times among aphidiine parasitoids, as suggested by an earlier study of oviposition time in aphidiine parasitoids (Völkl and Mackauer 2000). It is not clear from that study, however, whether oviposition time was influenced by host species (not identified). For instance, a species of parasitoid may take a long time to parasitize a host, not because the parasitoid is particularly inapt, but because the host may defend particularly well. The positive correlation between handling time and aphid body size in individual aphid-parasitoid association data and from aphid species data, on the other hand, may be related to the similarity amongst aphids. Contrary to aphidiine parasitoids, aphids generally lack specific adaptations that can affect the handling time of parasitoids independently of their body size. The efficacy of aphid physical defences such as kicking, swivelling or running away is likely positively related to body size. The excretion of cornicle secretions varies among species, but its use does not affect a parasitoid's handling time (Wu et al. 2010).

6.5.2 Comparative study of traits from interspecific interactions

Whereas comparative studies of foraging traits are often conducted from the consumer's perspective (Goss-Custard et al. 2006), our study reveals that such an approach may fail to reveal significant results. The analysis of parasitoid species data revealed no significant predictors of handling time, whereas aphid body length was related to handling time in the analyses of aphid species data and of individual aphid-parasitoid associations data. We suspect that using means of parasitoid species effectively eliminated some of the variance in aphid species body size, and thus reduced its explanatory power. Analysing individual consumer-resource species associations had the problem of ignoring phylogenetic relations between species, because standard tests of correlated evolution such as the phylogenetic least squares regression and phylogenetic independent contrasts use a single phylogeny (Freckleton et al. 2002; Garland et al. 2005). Here, we used a phylogenetic bipartite model (Ives and Godfray 2006) to account for the phylogenies of both consumers and resources without losing the information contained in individual relationships.

PREFACE TO CHAPTER 7

The previous chapter suggested that relative body size is not a major determinant of body size in communities of aphid parasitoids. This chapter investigates the body size relationship of parasitoids and aphids in a large community to determine whether it is consistent with size-based host preferences by parasitoids. In addition, it uses phylogenetic information to suggest evolutionary explanations for the body size relationships in aphid-parasitoid communities.

CHAPTER 7

PHYLOGENY EXPLAINS BODY SIZE RELATIONSHIPS IN APHID-PARASITOID ASSOCIATIONS

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(Chapter to be submitted to Evolutionary Ecology Research)

7.1 Abstract

The body size of consumers and their resources are often positively correlated and this relationship has often been attributed to ecological processes such as body size-dependent foraging behaviour shaping trophic interactions. Increasingly, evolutionary history (phylogeny) is shown to explain trophic interactions, so it could also explain the consumer-resource body size relationship. We conducted a phylogenetic study to determine whether phylogeny explains the consumer-resource body size relationship in aphid-parasitoid associations. Unique associations (n=418) between 48 parasitoid species (Hymenoptera, Braconidae, Aphidiinae) and 166 aphid species (Hemiptera, Aphididae) showed a significant positive correlation between parasitoid and host body sizes when ignoring phylogeny. When accounting for the phylogenies of both parasitoids and hosts, however, no significant correlation was found. This suggests that the body size relationship between aphids and parasitoids can be attributed to their evolutionary histories. Further analyses of phylogenetic patterns suggest that host size use diverged more than expected by chance through the evolutionary history of parasitoids. This result is consistent with disruptive selection of host size use by parasitoids having occurred in the past. This is the first study to investigate the body size relationship of consumers and their resources using phylogenies of both trophic levels, and shows that the body size relationship in aphid parasitoid systems can be completely attributed to their phylogenies.

Keywords: body size; parasitoid-host; community structure; phylogenetic signal

7.2 Introduction

7.2.1 Body size in foraging interactions

The body size of consumers and their associated resources are correlated in many communities including many predators and parasitoids, but not parasites or herbivores systems (Petchey et al. 2008). The conspicuousness of this relationship in an increasing number of communities is a strong indication of the importance of body size in shaping trophic interactions. Body size is related to many life history traits of animals, such as metabolic rate, longevity, and reproductive rate (Peters 1983) so that many size-dependent processes could influence trophic interactions. Abundance is also (inversely) related to body size in many animals, but possibly not so for small "r-selected" animals whose abundances are chaotic (Woodward et al. 2005). Most hypotheses point towards body size-dependent foraging; this includes body size-based niches, feeding hierarchies and explicit optimal foraging models (Beckerman et al. 2006; Petchey et al. 2008). For most predators, the ability to capture and subdue mobile or defending prey is dependent on their relative body size (Caro 2005; Griffiths 1980b). Thus, attacking a large prey may be easy for a predator of similar or larger size but too difficult for a smaller predator. Small predators may therefore be unable to attack large prey or choose not to attack them because of their high cost. Conversely, very small prey may be difficult for large predators to handle or be ignored because of their low value. The size of prey in the diet of optimally foraging predators can therefore be expected to increase with the forager's body size. While positive body size relationships seem generalized in predator-prey and parasitoid-host systems where prey or hosts can defend themselves, size-dependent foraging is not expected to produce this relationship in animals that do not physically subdue their prey or hosts such as herbivores and parasites, or those that hunt in groups.

When predators and parasitoids (consumers) that forage solitarily exploit multiple species of prey and hosts (resources), body size-dependent foraging can therefore influence resource species range. A simple extension of foraging theory would predict that large species of consumers tend to exploit large species of resources as they do when choosing resource size within species (see examples above). It follows that the body size of associated consumer and resource species should be positively correlated in ecological communities. While this prediction is simplistic, explicit foraging-based models of communities make similar predictions (Beckerman et al. 2006; Petchey et al. 2008).

7.2.2 Phylogeny

In addition to body size-dependent foraging and other ecological processes, evolutionary history (phylogeny) could contribute to the pattern of consumer-resource body sizes in present feeding relationships. The diet of an animal constitutes a fundamental part of its niche and may be phylogenetically conserved (Cavender-Bares et al. 2009; Webb et al. 2002; Wiens and Graham 2005). Consequently, phylogeny may determine the diet of consumers and feeding relationships in food webs, at least in part. In addition, body size is often phylogenetically conserved (Ashton 2004; Blomberg et al. 2003; Freckleton et al. 2002), so that pseudo-replication is likely a problem when inferring the body size relationship of closely related species (Felsenstein 1985; Freckleton et al. 2002; Harvey and Pagel 1991). Accounting for the phylogenies of animals in feeding relationships avoids these problems and can provide further insight into the evolutionary processes that generate the observed patterns (Cavender-Bares et al. 2009; Rezende et al. 2009). If resource size was mainly determined by phylogenetic conservatism in body size and diet of consumers, one would expect a positive phylogenetic signal (Blomberg et al. 2003), where closely related species of consumers exploit closely related species of resource with similar body sizes. Alternatively, frequency-dependent competition can favour the use of extremes in resource sizes. When accompanied by reproductive isolation, this can lead to disruptive selection (Futuyma and Moreno 1988), where resource size diverges during speciation events. Repeated events can lead to the diversification of resource size preferences over evolutionary time (Rueffler et al. 2006) and result in a negative phylogenetic signal (Blomberg et al. 2003). If large individuals are more efficient than small individuals at exploiting large resources, selection may favour the correlated evolution of body size and resource size preference in consumers (Troost et al. 2008). Moreover, observed consumer-resource body size relationships are the result of feeding relationships and should therefore depend on traits of consumers as well as traits of potential resources. Components of feeding relationships such as foraging rate can therefore depend on the phylogenies of both consumers and resources as shown recently in parasitoids of leafminers (Ives and Godfray 2006).

Body size relationships are often studied in symmetric food webs, where each species is assumed to be able to feed on every other species, albeit at different efficiencies (Brose et al. 2006; Cohen et al. 2003; Petchey et al. 2008; Warren and Lawton 1987). In asymmetric food webs, animals belong to clearly defined consumer or resource trophic levels, such that species of consumers feed on species of resources and not the reverse, and includes common interactions such as carnivore-herbivore or parasitoid-host interactions. A positive consumer-resource body size relationship is also predicted in asymmetric food webs when handling efficacy is dependent on consumer:resource body size ratio (Troost et al. 2008). For instance, food webs of certain freshwater fish (Layman et al. 2005) parasitoid-host (Cohen et al. 2005), or carnivores-herbivores

(Radloff and Du Toit 2004) are asymmetric and show a positive relationship between consumer and resource body sizes. Studying asymmetric systems reduces the complexity of food web interactions and permits the use of bipartite models to account simultaneously for the phylogenies of consumer and resource species (Ives and Godfray 2006).

7.2.3 Objective

The aim of this study was to test the hypothesis that phylogeny explains the positive body size correlation in consumer-resource associations. We examined the body size relationship between aphids (Hemiptera, Aphidoidea) and their common primary parasitoids (Hymenoptera, Braconidae, Aphidiinae) to: i) quantify the relationship; ii) test for the presence of phylogenetic signal in aphid and parasitoid body sizes with respect to the phylogeny of both; and iii) determine whether phylogeny explains the relationship between parasitoid and aphid body sizes. Aphid-parasitoids associations are well suited to these objectives for several reasons. First, behavioural studies in single aphid-parasitoid associations suggest that foraging behaviour may generate a positive relationship between consumer and resource body sizes. The body sizes of both aphids (Barrette et al. 2009; Chau and Mackauer 2001a; Hofsvang and Hågvar 1986; Weisser 1994) and parasitoids (Henry et al. 2009; G-M Wu et al unpublished) affect handling time and host size preference of parasitoids . Second, focussing on such a fairly homogeneous system also reduces the number of potentially confounding factors (Garland et al. 2005). Finally, the asymmetric nature of parasitoid-host feeding relationships also provides a simple system, which facilitates the use of phylogenetic methods (bipartite model) that account for the phylogenies of all protagonists (Ives and Godfray 2006).

7.3 Methods

7.3.1 Aphid-parasitoid associations

We analyzed feeding relationships between aphids and their primary parasitoids from a large compilation of surveys conducted in southeastern Europe over 13 years (Kavallieratos et al. 2004). The data consists of 32 208 individuals collected in 464 unique species associations between 176 aphid species and 97 parasitoid species. The large sample should provide a good representation of the feeding relationships in this system and location. Nevertheless, we excluded 49 species of “specialist” parasitoids (found on a single species of aphid). In addition to providing little information, specialist parasitoids were generally represented by a single or very few specimens. Such rare parasitoids may not provide an adequate picture of the aphid species associated with them. Analyses were based on the remaining 418 unique species associations between 166 species of aphids and 48 species of primary parasitoids.

7.3.2 Body size

We obtained species average body lengths of aphids and parasitoids from the literature. Aphid body lengths were mostly obtained from the extensive work of Blackman and Eastop (1994; 2006b) and completed with other publications. Parasitoid body lengths were compiled from taxonomic records and descriptions of new species as no single source covered all the species included in this study. Measures of body lengths were log-transformed for statistical analyses.

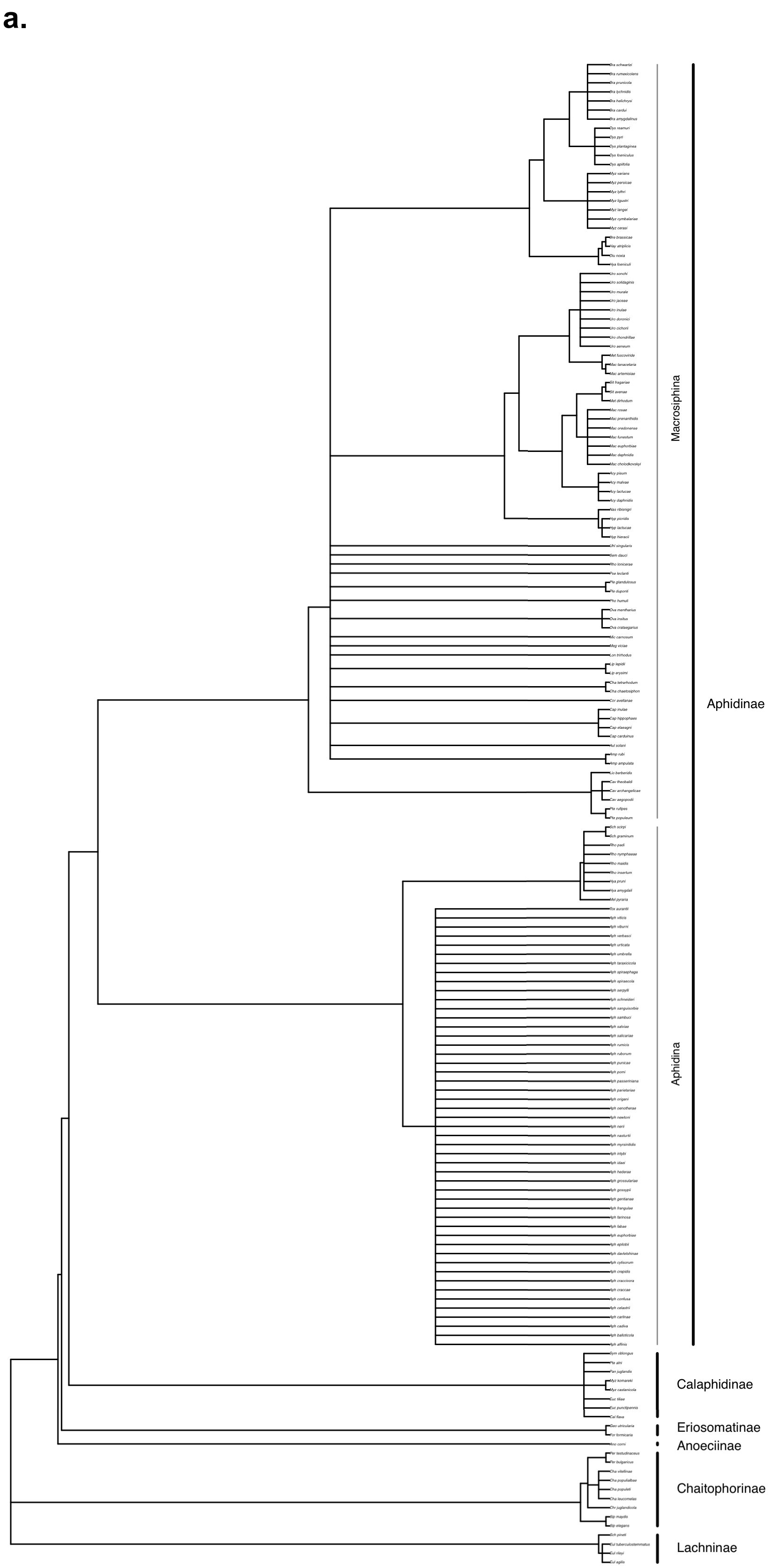
7.3.3 Phylogenies

The phylogenies of aphids (Figure 7.3.1a) and parasitoids (Figure 7.3.1b) were assembled using the following method. Published molecular phylogenies were combined into a composite tree, with conflicting branching patterns left unresolved (polytomies). Each genus was also left as unresolved because non-conflicting information on the branching patterns at the species level was seldom available. The resulting trees therefore captured only part of the phylogeny of the animals, so that the importance of phylogeny may be slightly underestimated in our study. Because branch lengths among phylogenies constructed from different molecular sequences are not comparable, the assembled trees were given arbitrary branch lengths following Grafen (1989).

7.3.4 Statistical analyses

The relationship between aphid and parasitoid body sizes was first estimated using an ordinary least squares regression (OLS) of aphid body length on parasitoid body length, and using a weighted least squares regression to give more weight to the most frequent feeding relationships (weight = log(n+1), where n = number of observed individual). These analyses implicitly assume no effect of evolutionary history on the body size of aphids in aphid-parasitoid associations (analyses of tip data).

A second analysis was then conducted to estimate the aphid and parasitoid body size relationship while accounting for the phylogenies of aphids and parasitoids simultaneously, using the phylogenetic bipartite linear model (Ives and Godfray 2006). The analysis was conducted under the Brownian Motion (BM) (Felsenstein 1985) and Ornstein-Uhlenbeck (OU) (Martins et al. 2002) models of trait evolution to test the robustness of the result. The BM model assumes completely random changes in trait evolution, whereas the OU model simulates random change within constrains such as a lower boundary in body size, or change around an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Uro jaceae
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Uro cichorii
Uro chondrillae
Uro aeneum
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Mac tanacetaria
Mac artemisiae
Sit fragariae
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Mac rosae
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Mac euphorbiae
Mac daphnidis
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Nas ribisnigri
Hyp picridis
Hyp lactucae
Hyp hieracii
Uhl singularis
Sem dauci
Rho lonicerae
Pse leclanti
Ple glandulosus
Ple duponti
Pho humuli
Ova mentharius
Ova insitus
Ova crataegarius
Mic carnosum
Meg viciae
Lon trirhodus
Lip lepidii
Lip erysimi
Cha tetrarhodum
Cha chaetosiphon
Cor avellanae
Cap inulae
Cap hippophaes
Cap elaeagni
Cap carduinus
Aul solani
Amp rubi
Amp ampulata
Lio berberidis
Cav theobaldi
Cav archangelicae
Cav aegopodii
Pte rufipes
Pte populeum
Sch scirpi
Sch graminum
Rho padi
Rho nymphaeae
Rho maidis
Rho insertum
Hya pruni
Hya amygdali
Mel pyraria
Tox aurantii
Aph viticis
Aph viburni
Aph verbasci
Aph urticata
Aph umbrella
Aph taraxicicola
Aph spiraephaga
Aph spiraecola
Aph serpylli
Aph schneideri
Aph sanguisorbie
Aph sambuci
Aph salviae
Aph salicariae
Aph rumicis
Aph ruborum
Aph punicae
Aph pomi
Aph passeriniana
Aph parietariae
Aph origani
Aph oenotherae
Aph newtoni
Aph nerii
Aph nasturtii
Aph myrsinitidis
Aph intybi
Aph idaei
Aph hederae
Aph grossulariae
Aph gossypii
Aph gentianae
Aph frangulae
Aph farinosa
Aph fabae
Aph euphorbiae
Aph epilobii
Aph davletshinae
Aph cytisorum
Aph crepidis
Aph craccivora
Aph craccae
Aph confusa
Aph celastrii
Aph carlinae
Aph cadiva
Aph balloticola
Aph affinis
Sym oblongus
Pte alni
Pan juglandis
Myz komareki
Myz castanicola
Euc tiliae
Euc punctipennis
Cal flava
Geo utricularia
For formicaria
Ano corni
Per testudinaceus
Per bulgaricus
Cha vitellinae
Cha populialbae
Cha populeti
Cha leucomelas
Chr juglandicola
Sip maydis
Sip elegans
Sch pineti
Eul tuberculostemmatus
Eul rileyi
Eul agilis
Macrosiphina
Aphidina
Aphidinae
Calaphidinae
Eriosomatinae
Anoeciinae
Chaitophorinae
Lachninae

b.

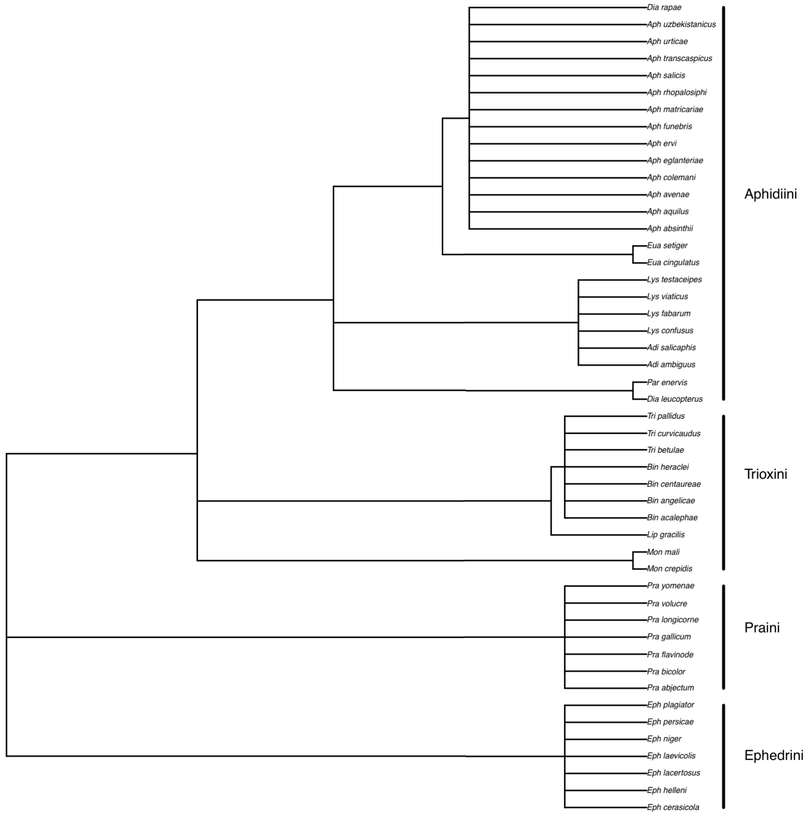


Figure 7.3.1 Topology of the phylogenetic trees for 166 species of aphids (a) and 48 species of parasitoids (b) included in analyses. High-resolution images of both phylogenies with species names are available on request.

optimal trait value. Analyses were also conducted using a star phylogeny (lack of phylogenetic dependence) to verify that any difference between the analysis of tips data (OLS) and phylogenetic analysis was not a bias from the statistical method.

Finally, we determined the pattern of evolutionary change using the statistic “d” for phylogenetic signal, for which d=0 suggests no phylogenetic dependence, 0<d<1 represents stabilizing selection, d=1 corresponds to random evolutionary change, and d>1 corresponds to disruptive selection (Blomberg et al. 2003; Ives and Godfray 2006).

Confidence intervals for the phylogenetic signal were computed using 1000 bootstrap samples. Statistical analyses were performed in R version 2.9.1 (R Development Core Team 2009), with the package “ape” version 2.3 (Paradis et al. 2004) for phylogenetics. The phylogenetic bipartite linear model and estimations of phylogenetic signal were all done using the package “picante” version 0.7.0 (Kembel et al. 2009). The function pblm() for the phylogenetic bipartite linear model was modified to accommodate missing feeding relationships (code available on request) The threshold of significance was set to 0.05 for all statistical tests.

7.4 Results

The body length of aphid species ranged from 0.85mm to 4.1mm (Figure 7.4.1a), and that of parasitoid species ranged from 1.1mm to 3.15mm (Figure 7.4.1b).

The body lengths of aphid species and parasitoid species were positively correlated (Figure 7.4.2) with a slope of 0.49 ($F=72.92$, $df=416$, $p<0.0001$, $R^2=0.15$). Weighted least squares regression also showed a highly significant relationship but with a slope of 0.62 ($F=112.2$, $df=416$, $p<0.0001$, $R^2=0.21$).

When accounting for the phylogenies of aphids and parasitoids, however, the relationship between aphid and parasitoid body size was no longer significant under either model of trait evolution (OU: $Z=0.056$, $p=0.5224$; BM: $Z=0.922$, $p=0.8218$). Using the star phylogeny, the bipartite model recovered the significant correlation as expected, with an identical slope of 0.48 ($Z=7.899$, $p<0.0001$).

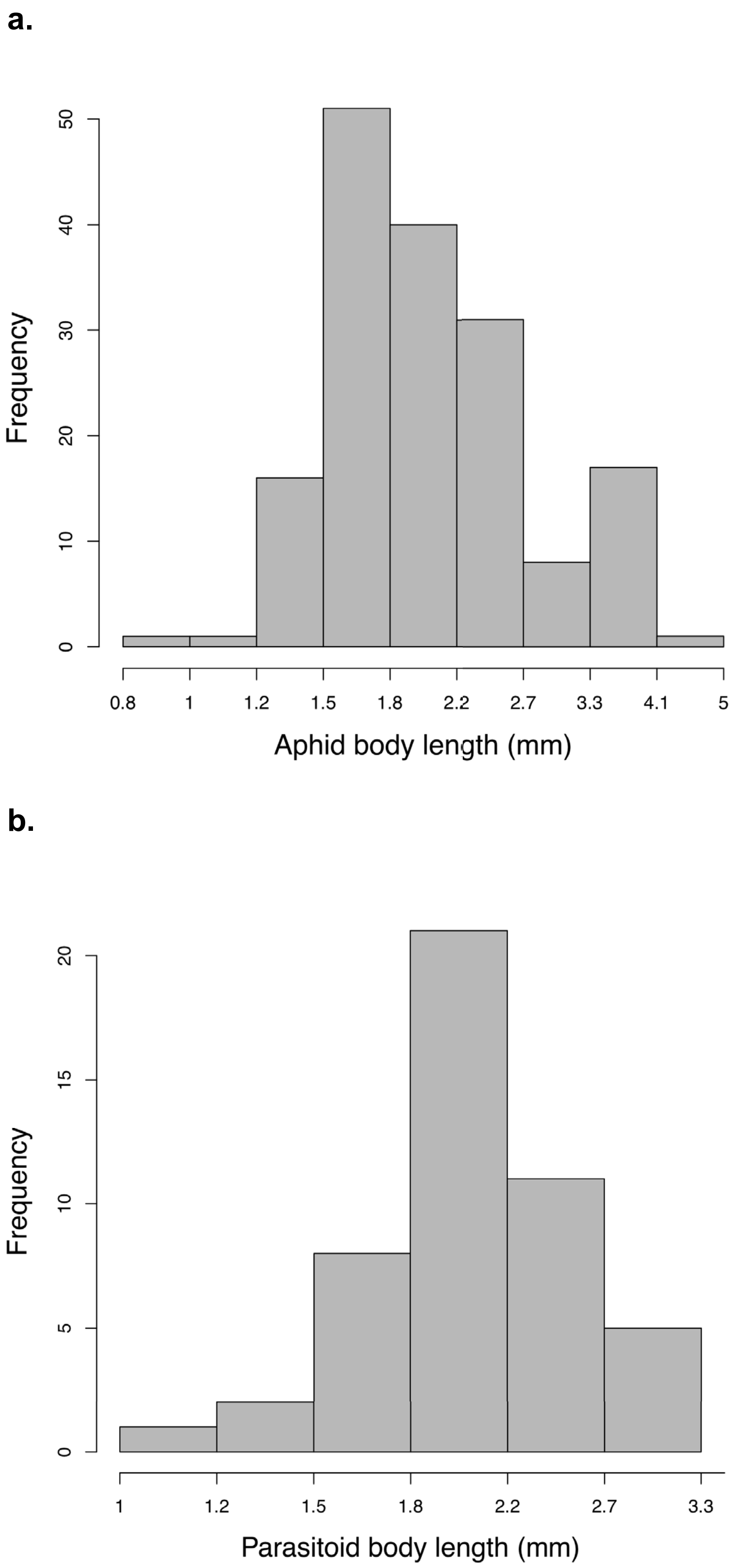


Figure 7.4.1 Frequency distributions of body lengths (log scale) for species of aphids (a) and parasitoids (b) included in the study.

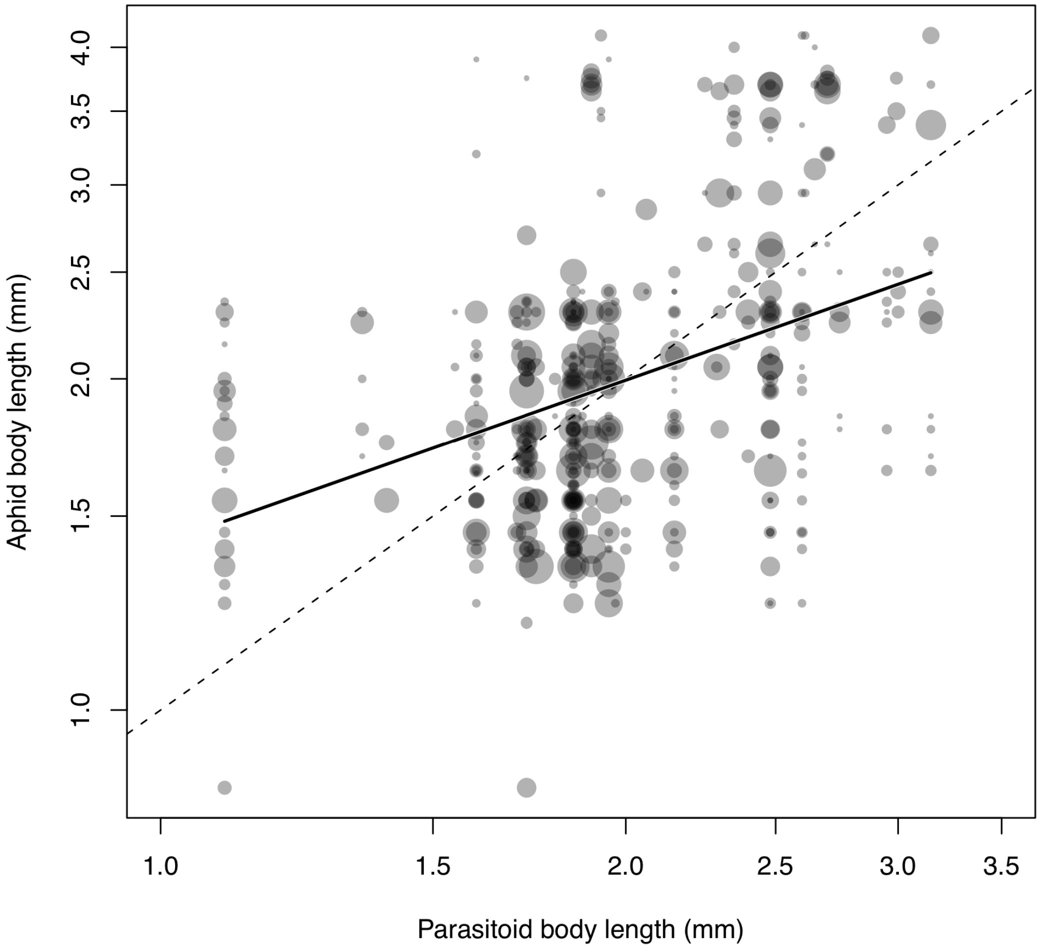
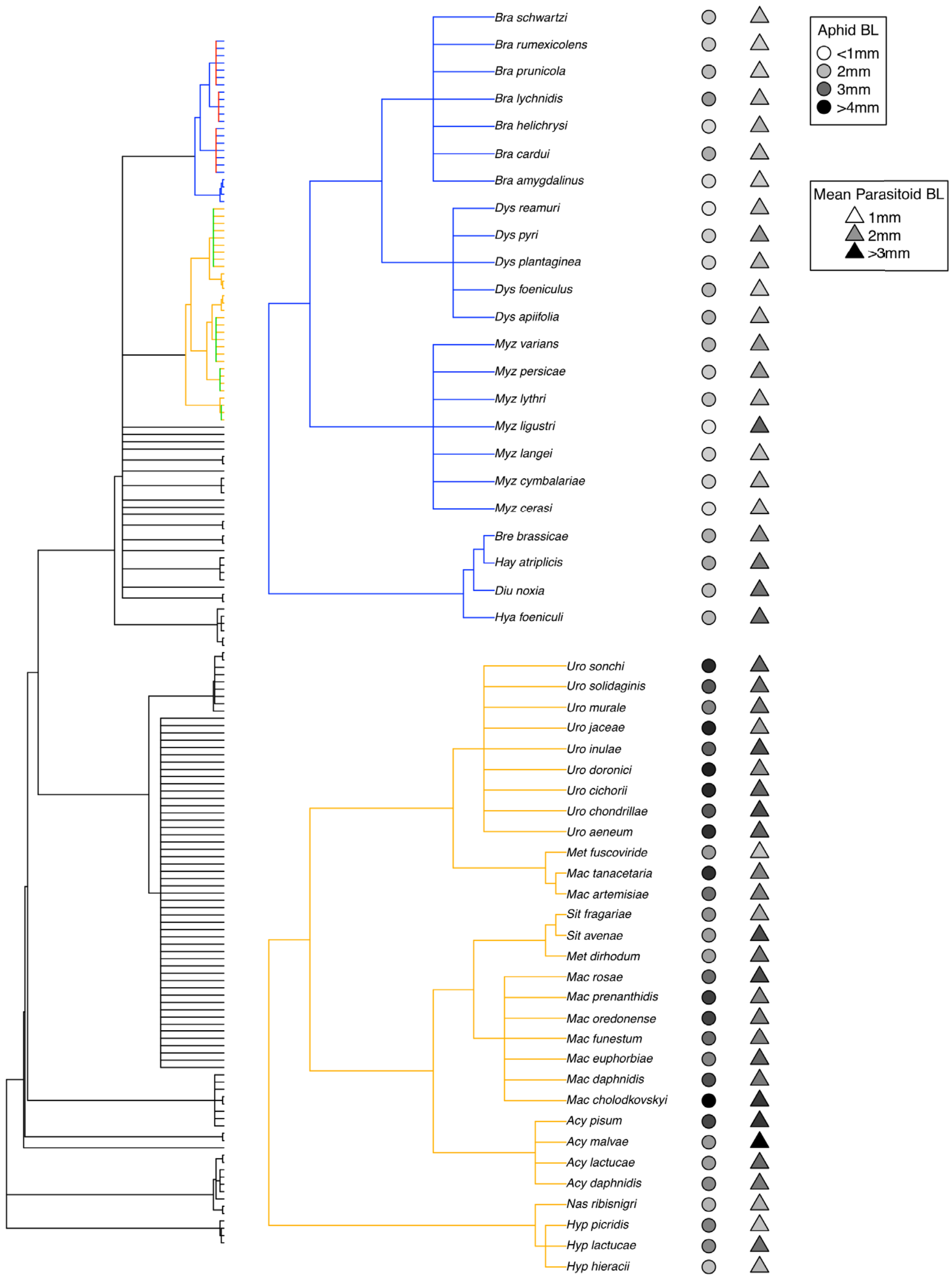


Figure 7.4.2 Relationship between aphid parasitoid size and host size use in feeding relationships (log-log scale). The solid line shows the fitted least squares linear regression (slope=0.49) and the dashed line shows the relationship for equal aphid and parasitoid body sizes (slope=1).

The body size of aphids in feeding relationships showed a phylogenetic signal with d = 0.209, which differed significantly from 0 (no phylogenetic signal) and 1 (random evolution). This suggests that aphid body size tends to be conserved through the phylogeny of aphids (Figure 7.4.3a). With respect to the phylogeny of parasitoids, aphid body size showed a phylogenetic signal with d = 4.210, which was significantly greater than 1 and consistent with disruptive selection (Figure 7.4.3b).

a.


Bra schwartzi
Bra rumexicolens
Bra prunicola
Bra lychnidis
Bra helichrysi
Bra cardui
Bra amygdalinus
Dys reamuri
Dys pyri
Dys plantaginea
Dys foeniculus
Dys apiifolia
Myz varians
Myz persicae
Myz lythri
Myz ligustri
Myz langei
Myz cymbalariae
Myz cerasi
Bre brassicae
Hay atriplicis
Diu noxia
Hya foeniculi
Uro sonchi
Uro solidaginis
Uro murale
Uro jaceae
Uro inulae
Uro doronici
Uro cichorii
Uro chondrillae
Uro aeneum
Met fuscoviride
Mac tanacetaria
Mac artemisiae
Sit fragariae
Sit avenae
Met dirhodum
Mac rosae
Mac prenanthidis
Mac oredonense
Mac funestum
Mac euphorbiae
Mac daphnidis
Mac cholodkovskyi
Acy pisum
Acy malvae
Acy lactucae
Acy daphnidis
Nas ribisnigri
Hyp picridis
Hyp lactucae
Hyp hieracii
Aphid BL
○ <1mm
● 2mm
● 3mm
● >4mm
Mean Parasitoid BL
△ 1mm
▲ 2mm
▲ >3mm

b.

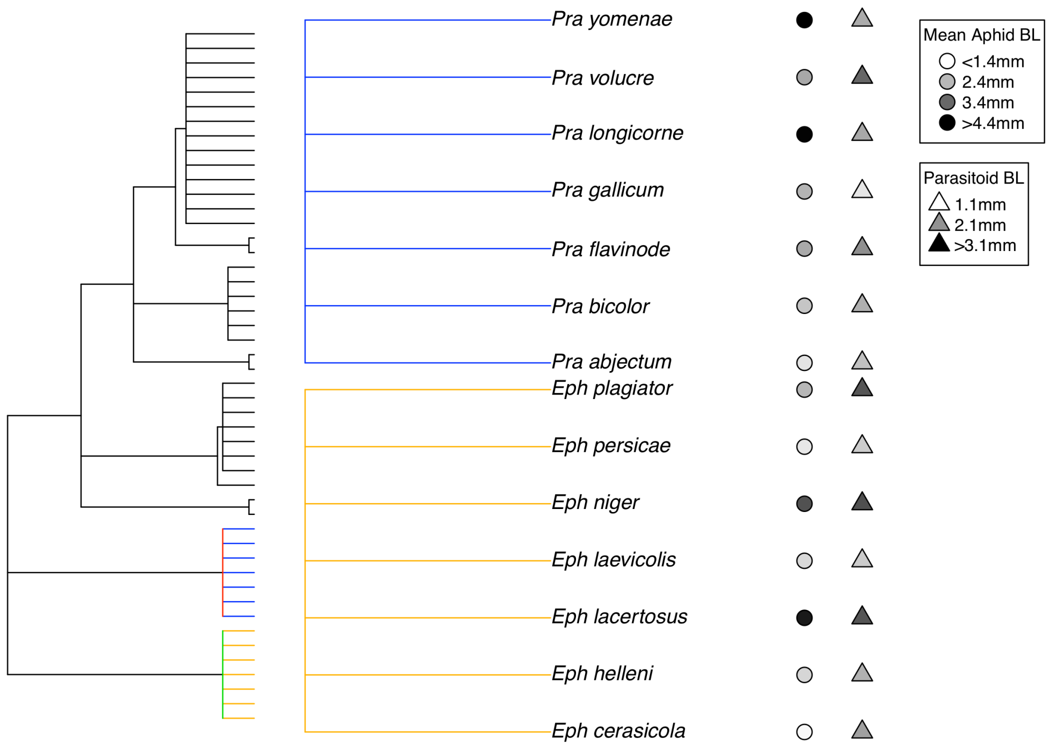


Figure 7.4.3 Phylogenetic patterns of aphid and parasitoid body sizes in feeding relationships. Body sizes of aphids (circles) and parasitoids (triangles) are shown for two clades of aphid and parasitoid phylogenies to illustrate the different phylogenetic patterns. Phylogenetic conservatism in aphid evolution (a): Closely related species of aphid share similar body sizes and are attacked by parasitoids of similar body sizes. Disruptive selection in parasitoid evolution (b): The body size of parasitoids and their respective hosts differ more within genera than between distant genera.

7.5 Discussion

The body sizes of aphids and parasitoids were positively correlated as in most consumer-resource associations, but the relationship could be attributed to the evolutionary history, completely so in our study. Further, the phylogenetic signals in host size suggest that body size is conserved in aphid evolution, and is consistent with disruptive selection in host size use by parasitoids. We discuss these results with respect to proposed mechanisms for consumer-resource body size relationship and for phylogenetic patterns. We also discuss potential implications for other studies of body size relationships and for food web dynamics.

7.5.1 Potential ecological mechanisms

The simple OLS regression (non-phylogenetic) showed a positive relationship between aphid size and parasitoid size. This agrees qualitatively with the relationship reported for aphid-parasitoid associations within a single community (Cohen et al. 2005). Cohen and co-workers (2005) reported a positive relationship (slope=0.50) when regressing the body size of primary parasitoid vs aphid body size. Despite the use of literature values, our OLS regressions of aphid size vs parasitoid size yielded a slope of 0.49; regressing parasitoid size vs aphid size using our data yielded a lower slope of 0.30 (analysis not shown). The positive parasitoid-aphid body size relationship is consistent with predictions from simple extensions of foraging theory and from explicit foraging-based models of food webs. Cohen and co-workers (2005), however, proposed that for aphid parasitoids, the relationship was due to physiological limitations of host size on the growth of parasitoids. For both foraging and physiological hypotheses, the positive relationship between parasitoid and aphid body size by itself, only constitutes circumstantial evidence.

Experiments in single aphid-parasitoid systems suggest that size-dependent foraging may be the cause, because the handling capacity of parasitoids increases with parasitoid:host body size ratio (Henry et al. 2009; Wu et al. 2011), and preferred host size increases with parasitoid body size (Henry et al. 2006). When comparing handling time across different aphid and parasitoid species associations, however, handling time varies with host size only and not parasitoid:host body size ratio (Chapter 6). Similarly, experimental investigations in single aphid-parasitoid systems support the physiological limitation hypothesis, but there is no evidence that this is also applicable in the context of different aphid-parasitoid species associations. Factors such as interspecific differences in host nutritional value, immunological response, or the presence of symbiotic bacteria (Cheng et al. 2010) can greatly affect the size of emerging parasitoids. Experiments on the parasitoid *Lysiphlebus testaceipes* and six different species of aphids (three Aphidini and three Macrosiphini) show that interspecific differences in host size are not correlated to the size of emerging parasitoids (Silva et al. 2008a). In the latter study, the three species of aphids belonging to the tribe Aphidini yielded the largest emerging parasitoids and the highest percentage of mummified hosts, a proxy for host preference. The three species of Macrosiphini on the other hand, yielded small or no parasitoids and few mummies. Physiological limitations of host size on parasitoid size may not be as important as other interspecific differences such as immunological defences or nutrition. This may be especially true for koinobiont parasitoids whose hosts may continue feeding during parasitoid development.

7.5.2 Contribution of evolutionary history

The lack of correlation between aphid and parasitoid body sizes in phylogenetic analyses suggests that the observed body size relationship can be attributed to phylogeny, completely so in our case. This suggests that the relationship is due to past evolutionary processes (Price 1997). For instance, parasitoid size dependent foraging may have been adaptive in the past and given rise to the positive body size relationship. A subsequent change in the environment or evolution of aphid parasitoids may change the selection regime so that size-dependent foraging is no longer selected for. As a result, phylogenetic inertia may be the only process maintaining the relationship (Losos 1996). In the case of aphidiine parasitoids, host searching is tightly linked to olfactory cues so that host range is likely constrained to aphids of certain plants. Aphid parasitoids have evolved different oviposition strategies such as the use of venom or specialized prongs that may allow them to get around the constraints of body size on handling capacity (Völkl and Mackauer 2000). The selective pressure on size-dependent foraging preferences may therefore be reduced. The lack of relationship between interspecific variation in parasitoid size and handling time is also consistent with this idea.

This result alone cannot establish whether current or past processes cause the observed body size relationship in aphid-parasitoid associations, but at the very least, it confirms the need for phylogenetic methods when investigating patterns in community ecology (Felsenstein 1985; Freckleton et al. 2002; Harvey 1996) such as body size relationships. The result was independent of the model of trait evolution used (Ornstein-Uhlenbeck or Brownian Motion), which suggests that it is robust. Further, the result is not a bias of the analytical method, because conducting the same analysis with a star phylogeny (assumption of no shared evolutionary history) gave the same result as the analysis of tips data (non-phylogenetic). Our phylogenetic analyses relied on phylogenetic trees that contained polytomies and arbitrary branch lengths, so the picture is likely only approximate. However, the use of even partial phylogenetic information is more accurate than assuming (implicitly) a star phylogeny. Despite the low resolution of the phylogenetic trees used in our study, a strong phylogenetic signal was revealed and fully resolved phylogenies may yield an even stronger result. Increasing the number of species for which common molecular sequences are available will likely provide more accurate phylogenetic trees. Sequencing many genes will be equally important as different genes yield conflicting topologies; the phylogenetic tree used in our study was conservative in this respect, because conflicting branching patterns were left unresolved. The dataset that was used comprised species associations at the "regional" scale (Kavallieratos et al. 2004), so that the observed phylogenetic pattern may differ at different spatial scales (Emerson and Gillespie 2008). It has been argued that phylogeny is less important at the level of "local" communities, where ecological processes are often thought to be the main drivers of community structure (Swenson et al. 2006). The aphid-parasitoid body size relationship revealed in our study was similar to the one found in a local community (Cohen et al. 2005). Furthermore, a similar relationship was found within habitats. Simple linear (OLS) regressions of aphid vs. parasitoid body sizes in each of the two habitats containing more than 10 species of parasitoids, "European deciduous forest" and "Eurasian Steppes" (Kavallieratos et al. 2004), also yielded highly significantly relationships (slope=0.22, $F_{(1,64)}=27.3$, $p<0.0001$, $R^2=0.29$, and slope=0.36, $F_{(1,252)}=44.7$, $p<0.0001$, $R^2=0.15$, respectively). The positive body size relationship is therefore not unique to the regional scale, and the loss of the relationship in phylogenetically controlled analyses is not likely due to habitat differences or the significant within habitat relationships would have been recovered. Phylogenetic analyses within habitats failed to converge so that we could not confirm directly that phylogeny explained body size relationships within habitats. Confirmation of this result would be consistent with the increasing recognition that both ecological and evolutionary processes can affect trophic interactions at all spatial scales (Emerson and Gillespie 2008; Ricklefs 1987; Ricklefs 2008; Vellend 2010).

7.5.3 Patterns of phylogenetic signal

7.5.3.1 Aphid phylogeny

Aphid body size was highly conserved through aphid phylogeny as found in many animals (Blomberg et al. 2003). This may be due to strong selective pressures that many life history traits place on body size. Whereas aphids could evolve larger body sizes to better defend against potential enemies, doing so incurs a longer development time and hence, a lower rate of population increase. The phylogenetically conserved body size of aphids has implications for inference on the consumer-resource body size relationships. Parasitoids that attack many aphid species of similar sizes may be selecting a clade of aphids rather than aphid size itself. One possible mechanism for this phylogenetically conserved diet is their use of plant chemical cues to find hosts (Godfray 1994; Vinson 1976). Because different plants harbour different taxa of aphids, a parasitoid's attraction towards a plant odour may translate to a preference for the clade of aphids (that are incidentally of similar sizes). In addition, there is experimental evidence that aphidiine parasitoids prefer aphids that are closely related (Desneux et al. 2009; Silva et al. 2008a). This likely explains why controlling for phylogeny yielded no significant relationship between parasitoid and host size. Resource phylogeny may also explain body size relationships in other food webs, because body size is phylogenetically conserved in many animals (Ashton 2004; Blomberg et al. 2003; Freckleton et al. 2002).

7.5.3.2 Parasitoid phylogeny

Closely related parasitoids tended to be associated with different sized aphids, which is consistent with disruptive selection on host size use. Disruptive selection is usually attributed to negative frequency-dependent payoffs, such that competition among parasitoids for a given host size selects for individuals that use different host sizes (Rueffler et al. 2006; Troost et al. 2008) or more generally, different niches (Emerson and Gillespie 2008). Alternately, disruptive selection can occur without diversification if there is an inherently maladaptive intermediate trait value (Rueffler et al. 2006). In the latter case, one would expect a bimodal distribution of parasitoid size and host size use, which is not what we found (Figure 7.3.1). Results were therefore more consistent with disruptive selection caused by negative frequency-dependent payoffs (Figure 7.4.3b). For frequency-dependent competition to cause disruptive selection, individuals with different traits must be reproductively isolated. This is likely the case in aphid parasitoids, because their life history is such that host selection determines in part, the mating site of the next generation. Aphidiine parasitoids are quasi-gregarious; females lay their eggs in aggregated hosts (Hardy 1994), so their offspring will emerge close to each other. Consequently, siblings tend to mate with each other and with offspring of conspecific females that expressed similar host preferences. Few other consumer-resource associations have been studied with phylogenetic methods. In a study of mostly asymmetric aquatic food webs, the body sizes of fish predators and their prey are positively correlated even when taking into account the phylogeny of predator species (Rezende et al. 2009). In the study of aquatic food webs, the phylogeny of prey was not accounted for. Alternately, prey size choice in fish may not result in reproductive isolation as it does for aphid parasitoids.

7.5.3.3 Combined aphid and parasitoid phylogenies

In their model of asymmetric food webs, Troost and colleagues (2008) assumed that prey had a fixed size distribution, and simulated the evolution of predator body size and prey size use. Our results suggest that this simplifying assumption is reasonable for aphid parasitoids, because body size tended to be phylogenetically conserved through the evolution of aphids (Figure 7.4.3a). In systems where the body size of both protagonists are not phylogenetically conserved, consumer body size is predicted to stabilize near the mean resource size, and resource size is predicted to undergo disruptive selection away from the mean consumer size (Rueffler et al. 2006), the opposite of our results.

7.6 Conclusions

One of the goals of community models is to predict changes in community structure in reaction to disturbances such as species extinctions, introductions of alien species, or changes in the environment. For strictly ecological models, this is only possible as far as the community is a dynamic outcome of current ecological processes such as foraging decisions by consumers. Patterns of community structure resulting from slow evolutionary change will be much slower to alter than those resulting from ecological processes. Consequently, the amount of phylogenetic signal in the structure of a community may indicate how fast it can react to disturbances (Cavender-Bares et al. 2009; Jablonski and Sepkoski 1996; Rezende et al. 2009). Our study suggests that evolutionary history (phylogeny) plays an important role in current for aphid parasitoid feeding relationships. These communities may therefore be vulnerable to disturbances.

CHAPTER 8

SUMMARY AND CONCLUSIONS

Most studies of diet choice and host choice have focussed on individual behaviour in single trophic interactions. While it has long been proposed that individual dietary choices affect the nature and strength of trophic interactions in ecological communities, few studies have provided empirical support for this hypothesis. In large ecological communities, body size (and associated handling cost) has been proposed as the common currency from which foragers establish their dietary preferences, such that body size relationships between consumer and resource species can provide evidence for the role of individual foraging behaviour in structuring ecological communities. This thesis showed that the relative body size of an aphid parasitoid influences its host handling efficiency (Chapter 3) and that individual parasitoids forage optimally for differently sized hosts of a given species (Chapter 5). However, handling efficiency among different species of parasitoids and aphids was not a function of relative parasitoid size (Chapter 6), and the correlation between parasitoid and host size was completely attributed to phylogeny (Chapter 7). This suggests that the current host range of parasitoids is determined by their evolutionary history, but is also consistent with optimal host choice having structured communities in the past. These results have implications for scaling up models of individual behaviour to larger levels of organisations; scaling down from evolutionary patterns to individual behaviour; and various aspects of applied ecology.

8.1 Scaling up from individual foraging behaviour

Handling cost plays a significant role in the profitability of resource items and has been shown to be negatively related to the consumer:resource body size ratio within many systems (Brose 2010). However, few studies have shown that this relationship can be scaled up to multi-species trophic interactions. Chapter 3 showed that the handling cost of *A. colemani* foraging for *M. persicae* is inversely related to parasitoid:host body size ratio as predicted by the relative body size hypothesis. This result is in agreement with a study in another aphid parasitoid, *A. ervi* (Henry et al. 2009). The relative body size hypothesis may therefore be generalized in aphid parasitoids with respect to intraspecific variation in body size (all else being equal). Moreover, preference for larger hosts increases with body size of aphidiine parasitoids (Henry et al. 2009; Lykouressis et al. 2009), providing evidence for the role of relative body size in host choice. Scaling up to the level of ecological communities, however, depends on interspecific variation in the body size of parasitoids and hosts. In addition, adaptations such as aphid cornicle secretions (Chapter 4) differ among species, so that body size may become secondary. At the level of communities, the phylogenetic comparative study of handling time (Chapter 6) did not support the relative size hypothesis, and instead suggests that handling time is a function of host size only. Resource size preference is therefore not expected to vary with the size of consumers so the body size of consumers and resources are not predicted to be correlated (Petchey et al. 2008). Accordingly, the analysis of parasitoid-host body size relationships (Chapter 7) showed no positive correlation between parasitoid and host body sizes when accounting for phylogeny. Phylogeny may therefore contribute to the structure of aphid-parasitoid communities more than body size-dependent host choices at present (Losos 1996). Choice experiments with multiple parasitoid and host species are needed for a stronger test of the effect of body size on host choice in communities. Whether the body size relationship arose through past size-dependent host choices or other mechanisms (Cohen et al. 2005), this thesis shows that models of aphid-parasitoid communities structure should include a phylogenetic component (Cattin et al. 2004) or model evolution explicitly (Troost et al. 2008). The influence of phylogeny was also shown in the strength of interactions in leafminer parasitoids (Ives and Godfray 2006) and compartmentalization in an aquatic predatory system (Rezende et al. 2009). In major classes of lizards, most differences in diet types can be attributed to evolutionary history rather than present day ecological interactions (Vitt and Pianka 2005). Nevertheless, a positive correlation between predator size and prey size remains. However, only the study of leafminers (Ives and Godfray 2006) and this thesis have considered the phylogenies of consumers and resources.

8.2 Scaling down to individual foraging behaviour

This thesis explored how individual dietary choices can scale up to higher-level processes, and I argue that the reverse may be as valuable: evolutionary and community level studies could be “scaled down” to better understand individual diet choices (Figure 8.2.1). Phylogenetic constraints should be expressed at the level of the individual and may affect our interpretation of observed diet choices. For instance, the aphid parasitoid *Lysiphlebus testaceipes* prefers the three species that are taxonomically closest to its native host when presented with six species of aphids (Silva et al. 2008a). Without the insight from phylogeny, the understanding of diet choices or other behaviours can be incomplete or inaccurate (Ryan 2005).

The effects of phylogenetic constraints on individual dietary choices may depend on the proximate mechanisms that maintain these constraints and on the context in which dietary choices are studied. In many aphid parasitoids, initial host location is largely through olfactory cues associated with the aphid’s host plants (Vet 2001; Vinson 1976). If this was the principal mechanism for host choices, phylogenetic constraints would be expressed only when parasitoids are presented with aphid-plant complexes, and not with aphids alone. The use of plant olfactory cues is a good candidate mechanism for aphid parasitoids and many other insects, because of the prevalence of innate odour preferences in many insects (Steidle and van Loon 2003). Investigating the phylogenetic pattern of odour preferences may contribute to determine whether phylogenetic constraints in host range are mediated by olfaction.

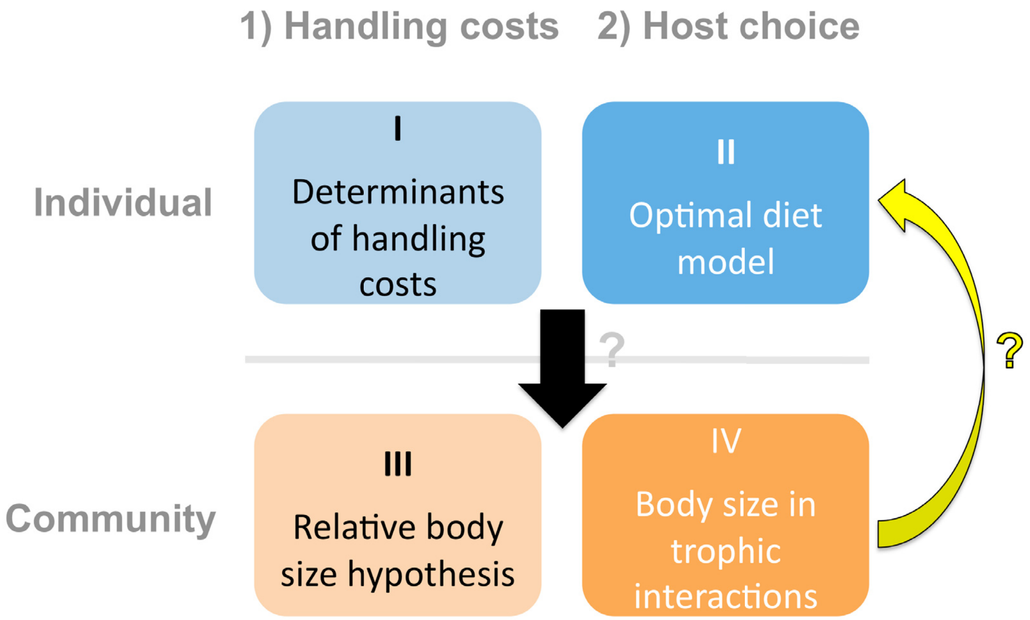


Figure 8.2.1 Diagram of the general questions addressed in this thesis and the scale at which they are investigated. Questions were addressed at the level of individual behaviour (blue) and communities (orange) to evaluate the implications of individual foraging behaviour for higher levels of organisation (black arrow). In the light of the findings, knowledge about the factor structuring communities, phylogenetic patterns, can be used to better understand individual diet choices (yellow arrow).

8.3 Implications for applied ecology

The influence of phylogeny on host range (Chapter 7) limits the capacity of parasitoids to adapt to changes in the environment. The optimal diet model and its extensions to population and community dynamics generally assume that animals are labile and can include different resource types when it is profitable to do so. While phenotypic plasticity may allow parasitoids to expand their host range or to switch host species quickly, phylogenetic constraints may make these changes only possible only through slower evolutionary processes. This has implications for the dynamics of ecological communities and aspects of applied ecology such as climate change, biological invasions and biological control.

Climate change will likely affect the body size of ectothermic animals because of the effect of developmental temperature on adult body size (Atkinson 1994; Atkinson and Sibly 1997; Kingsolver and Huey 2008) and therefore size-dependent foraging capacity (Chapter 3). Differential effects of temperature on consumer and resource species could alter their body size ratios and alter the ability of consumers to exploit their usual resources. Foragers could compensate for this difference by selecting smaller or larger individuals (e.g. instars) within the same species, but phylogenetic constraints may prevent them from compensating by using different species of resources.

New species of animals can invade a community following a change in climate, voluntary or accidental introductions. Predicting the effects of alien species on an ecological community may be useful to assess threats to native resource species or to identify biological control agents, which may consume the former. Ideally, one could study the full biology of each alien species, perform behavioural assays with all susceptible species, and perform mesocosm experiments. All this, however, may require more time and resource than is available. Phylogenetic information may help to reduce the number of experiments required and to select the most informative ones. Depending on the prevailing phylogenetic pattern found in a given clad, predicting an animal’s potential resource species may be easier than predicting its potential consumer species. For instance, hosts of a given parasitoid species are likely phylogenetically closely related, (Silva et al. 2008a), whereas parasitoids of a given aphid species may be phylogenetically very distant due to disruptive selection (Chapter 7).

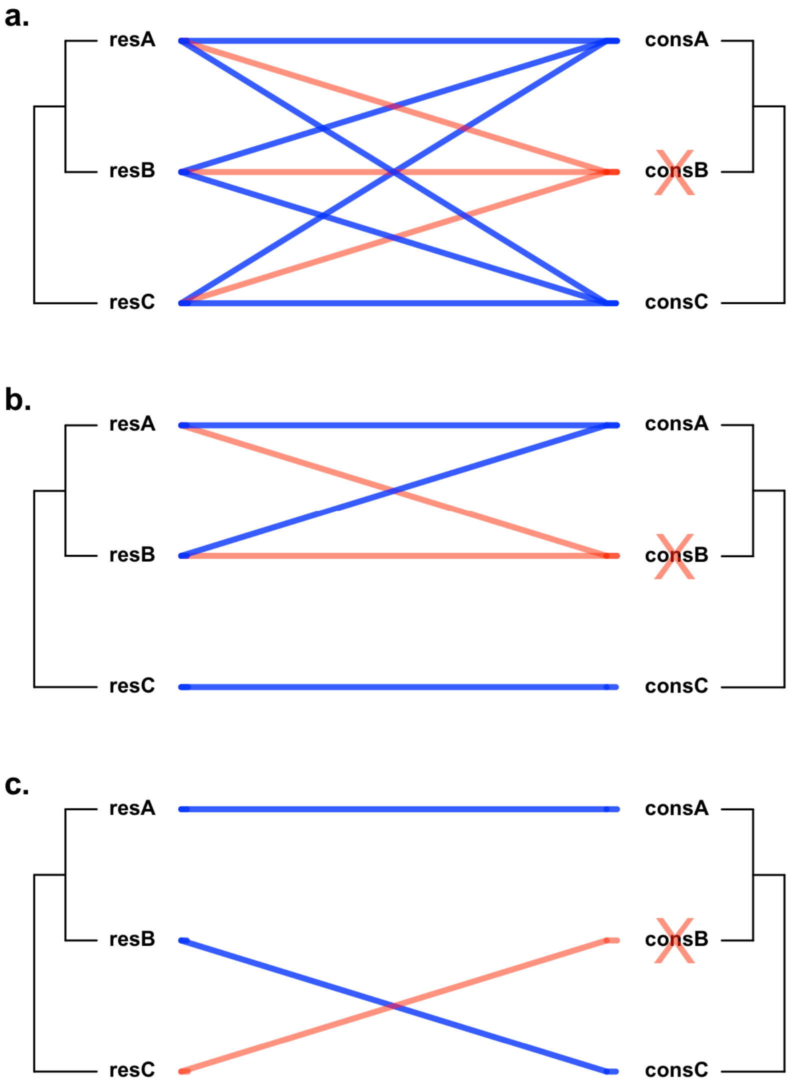


Figure 8.3.1 Trophic interactions between hypothetical resource (left) and consumer species (right) represented by connecting lines. Red lines show interactions that would cease if consumer B (consB) became extinct. When there is: a) no phylogenetic constraint, other consumers (consA, consC) can exploit resources freed; b) phylogenetic conservatism, closely related consumers (consA) can exploit freed resource; and c) disruptive selection, resource freed cannot be exploited by closely related consumers (consA).

Overexploitation of animals and environmental changes can lead species to extinction. The resulting impact on ecological communities may depend in part on the processes that structure of the community. In communities that are completely structured by individual dietary choices, functionally equivalent consumers can adjust their diets and fill the niche made available by extinct species (Figure 8.3.1a). In such communities, loss of biodiversity may be less likely to have a major effect on ecosystem functions. Other communities show evidence of compartmentalisation, with little interactions among different groups of consumers and resources. This can arise from phylogenetic conservatism of dietary choices by consumers (Rezende et al. 2009). In such communities, the loss of a species could be compensated by functionally similar species within the compartment, while species from other compartments may be relatively unaffected (Figure 8.3.1b). In aphid parasitoid communities, closely related genera of parasitoids consume very different species of aphids due to a pattern of disruptive selection (Figure 8.3.1c). Phylogenetic proximity may therefore not be correlated to functional similarity and extinction of seemingly redundant species may have significant effects on these communities.

8.4 Perspectives

Clearly, dietary choices both at the individual and community levels are multifaceted. For instance, interspecific differences in defence behaviours by resources such as cornicle secretions of aphids (Chapter 4), spines on potential prey or other adaptations modulate the dietary choices of consumers. This thesis focussed on the trade off between fitness gain and handling cost that host size represents for parasitoids, because it is the most universal determinant of resource profitability, and the most relevant for scaling up to the level of ecological communities.

One potential caveat is that body size is correlated to other traits so that patterns of body size relationships may be due to other traits correlated to body size. For instance, large host aphids may also express greater immunological responses, which may affect their fitness value. In experiments at the level of individuals, immunological cost of large hosts was accounted for implicitly in the measure of fitness gain (Chapter 5). At the level of communities, different species of aphid hosts present different degrees of immunological response, but there is little evidence that this is correlated to species body size. Interspecific differences in immunological responses may be more related to the presence of symbiotic bacteria (Oliver et al. 2003). Other potential mechanisms (Cohen et al. 2005) remain to be investigated using phylogenetic approaches.

The attribution of the parasitoid-aphid body size relationship to phylogeny highlighted the importance of considering phylogeny when studying multiple species. As body size shows a phylogenetic signal in most animals, this result is likely relevant to other systems. Moreover, the body size of prey or hosts is also expected to be phylogenetically dependent so that studies of body size relationships in other systems should also account for the phylogenies of both consumers and resources (Ives and Godfray 2006). Current phylogenetic methods may not allow this analysis in symmetric food webs, where each species may belong to many trophic levels, but the use of asymmetric predator-prey food webs may provide additional information; mainly as to whether the attribution of body size relationship to phylogeny is specific to parasitoids or other specific characteristics of the system used here.

Finally, the phylogenetic patterns of traits and consequently the body size relationship could be scale-dependent (Cavender-Bares et al. 2009). Comparative studies (Chapters 6 and 7) were conducted within parasitoids of the subfamily Aphidiinae, which all attack aphids (Aphididae), so the phylogenetic patterns are not due to obvious differences such as herbivory vs carnivory, aquatic vs terrestrial, etc. The trophic interactions studied (Kavallieratos et al. 2004) were drawn at the regional scale, but it is not clear how the influence of phylogeny would differ at smaller scales. The bipartite model could not be run for subsets of habitats to test this specifically, possibly because of the smaller number of species. However, the host range of parasitoids such as *Aphidius matricariae* that spans over 10 genera and 30 species of aphids on grasses, herbs and trees, suggests that there may be no major phylogenetic or spatial barrier to host range for at least some species, so that ecological processes could occur at this scale. At very large scales, trophic interactions may also be related to the phylogeny of plants (Ives and Godfray 2006), because of the close association between phytophagous insects and plants. With larger datasets (Brose et al. 2005) it may be possible to explore extensively the importance of scale, but it is beyond the scope of this thesis.

LITERATURE CITED

Abouheif E (1999) A method for testing the assumption of phylogenetic independence in comparative data. Evolutionary Ecology Research 1:895-909.

Akaike H (1971) Information theory and extension of the maximum likelihood principle. In: Petrov BN, Csáki F (eds) Second International Symposium on Information Theory. Akademiai Kiado, Tsahkadsor, Armenia, USSR, pp 267-281.

Akaike H (1974) A new look at the statistical model identification. IEEE Transactions on Automatic Control 19:716-723.

Aljetlawi AA, Sparrevik E, Leonardsson K (2004) Prey-predator size-dependent functional response: Derivation and rescaling to the real world. Journal of Animal Ecology 73:239-252.

Anderson DR, Burnham KP (2002) Avoiding pitfalls when using information-theoretic methods. Journal of Wildlife Management 66:912-918.

Angilletta MJ, Jr , Steury TD, Sears MW (2004) Temperature, growth rate, and body size in ectotherms: Fitting pieces of a life-history puzzle. Integrative and Comparative Biology 44:498-509.

Arim M, Bozinovic F, Marquet PA (2007) On the relationship between trophic position, body mass and temperature: reformulating the energy limitation hypothesis. Oikos 116:1524-1530.

Ashton KG (2004) Comparing phylogenetic signal in intraspecific and interspecific body size datasets. Journal of Evolutionary Biology 17:1157-1161.

Atkinson D (1994) Temperature and organism size - A biological law for ectotherms? In: Begon M, Fitter AH (eds) Advances in Ecological Research, vol 25. Academic Press, pp 1-58.

Atkinson D, Sibly RM (1997) Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. Trends in Ecology and Evolution 12:235-239.

Avery RA, Bedford JD, Newcombe CP (1982) The role of thermoregulation in lizard biology: Predatory efficiency in a temperate diurnal basker. Behavioral Ecology and Sociobiology 11:261-267.

Avery RA, Mynott A (1990) The effects of temperature on prey handling time in the common lizard, *Lacerta vivipara*. Amphibia-Reptilia 11:111-122.

Barrette M, Wu G-M, Brodeur J, Giraldeau L-A, Boivin G (2009) Testing competing measures of profitability for mobile resources. Oecologia 158:757-764.

Beckerman AP, Petchey OL, Warren PH (2006) Foraging biology predicts food web complexity. Proceedings of the National Academy of Sciences of the United States of America 103:13745-13749.

Begon M, Harper JL, Townsend CR (1996) Ecology: Individuals, populations and communities, 3rd edn. Blackwell Scientific, Cambridge, MA.

Belshaw R, Dowton M, Quicke DLJ, Austin AD (2000) Estimating ancestral geographical distributions: A Gondwanan origin for aphid parasitoids? Proceedings of the Royal Society Biological Sciences Series B 267:491-496.

Benrey B, Denno RF (1997) The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. Ecology 78:987-999.

Bernal JS, Gonzalez D (1997) Reproduction of *Diaeretiella rapae* on Russian wheat aphid hosts at different temperatures. Entomologia Experimentalis et Applicata 82:159-166.

Bersier L-F, Kehrli P (2008) The signature of phylogenetic constraints on food-web structure. Ecological Complexity 5:132-139.

Bicego KC, Barros RCH, Branco LGS (2007) Physiology of temperature regulation: Comparative aspects. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 147:616-639.

Blackman RL, Eastop VF (1994) Aphids on the World's Trees: An Identification and Information Guide. CAB International, Wallingford, UK.

Blackman RL, Eastop VF (2000) Aphids on the World's Crops: An Identification and Information Guide, 2nd edn. John Wiley & Sons Ltd, Chichester, UK.

Blackman RL, Eastop VF (2006a) Aphids on the World's Herbaceous Plants and Shrubs. Volume 1 Host Lists and Keys. John Wiley & Sons, Ltd, Chichester, England.

Blackman RL, Eastop VF (2006b) Aphids on the World's Herbaceous Plants and Shrubs. Volume 2 The Aphids. John Wiley & Sons, Ltd, Chichester, England.

Blanckenhorn WU (1991) Fitness consequences of foraging success in water striders *Gerris remigis* (Heteroptera, Gerridae). Behavioral Ecology 2:46-55.

Blomberg SP, Garland TJ, Ives AR (2003) Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. Evolution 57:717-745.

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.

Boivin G, Jacob S, Damiens D (2005) Spermatogeny as a life-history index in parasitoid wasps. Oecologia 143:198-202.

Braendle C, Davis GK, Brisson JA, Stern DL (2006) Wing dimorphism in aphids. Heredity 97:192-199.

Brandl R, Kristin A, Leisler B (1994) Dietary niche breadth in a local community of passerine birds: an analysis using phylogenetic contrasts. Oecologia 98:109-116.

Breed MD, Guzman-Novoa E, Hunt GJ (2004) Defensive behavior of honey bees: Organization, genetics, and comparisons with other bees. Annual Review of Entomology 49:271-298.

Brodeur J, Boivin G (2004) Functional ecology of immature parasitoids. Annual Review of Entomology 49:27-49.

Brodeur J, Geervliet JBF, Vet LEM (1996) The role of host species, age and defensive behaviour on ovipositional decisions in a solitary specialist and gregarious generalist parasitoid (*Cotesia* species). Entomologia Experimentalis et Applicata 81:125-132.

Brodeur J, Rosenheim JA (2000) Intraguild interactions in aphid parasitoids. Entomologia Experimentalis et Applicata 97:93-108.

Brose U (2010) Body-mass constraints on foraging behaviour determine population and food-web dynamics. Functional Ecology 24:28-34.

Brose U, Cushing L, Berlow EL, Jonsson T, Banasek-Richter C, Bersier L-F, Blanchard JL, Brey T, Carpenter SR, Cattin-Blandenier M-F, Cohen JE, Dawah HA, Dell T, Edwards F, Harper-Smith S, Jacob U, Knapp RA, Ledger ME, Memmott J, Mintenbeck K, Pinnegar JK, Rall BC, Rayner T, Ruess L, Ulrich W, Warren P, Williams RJ, Woodward G, Yodzis P, Martinez ND (2005) Body sizes of consumers and their resources. Ecology 86:2545-2545.

Brose U, Jonsson T, Berlow EL, Warren P, Banasek-Richter C, Bersier L-F, Blanchard JL, Brey T, Carpenter SR, Cattin-Blandenier M-F, Cushing L, Dawah HA, Dell T, Edwards F, Harper Smith S, Jacob U, Ledger ME, Martinez ND, Memmott J, Mintenbeck K, Pinnegar JK, Rall BC, Rayner TS, Reuman DC, Ruess L, Ulrich W, Williams RJ, Woodward G, Cohen JE (2006) Consumer-resource body-size relationships in natural food webs. Ecology 87:2411-2417.

Brown GE, Godin J-GJ (1999) Who dares, learns: chemical inspection behaviour and acquired predator recognition in a characin fish. Animal Behaviour 57:475-481.

Bueno RJ, van Cleave HW (1997) The effect of temperature and host density on the reproduction of *Aphelinus perpallidus*. Southwestern Entomologist 22:29-37.

Buitenhuis R, Boivin G, Vet LEM, Brodeur J (2004) Preference and performance of the hyperparasitoid *Syrphophagus aphidivorus* (Hymenoptera: Encyrtidae): fitness consequences of selecting hosts in live aphids or aphid mummies. Ecological Entomology 29:648-656.

Burnham KP, Anderson DR (2002) Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach, 2nd edn. Springer, New York.

Butler CD, O'Neil RJ (2006) Defensive response of soybean aphid (Hemiptera : Aphididae) to predation by insidious flower bug

(Hemiptera : Anthocoridae). Annals of the Entomological Society of America 99:317-320.

Byers JA (2005) A cost of alarm pheromone production in cotton aphids, *Aphis gossypii*. Naturwissenschaften 92:69-72.

Byström P, Andersson J, Kiessling A, Eriksson L-O (2006) Size and temperature dependent foraging capacities and metabolism: consequences for winter starvation mortality in fish. Oikos 115:43-52.

Callow RK, Greenway AR, Griffith DC (1973) Chemistry of secretion from cornicles of various species of aphids. Journal of Insect Physiology 19:737-748.

Cambier V, Hance T, De Hoffmann E (2001) Effects of 1,4-Benzoxazin-3-One derivatives from maize on survival and fecundity of *Metopolophium dirhodum* (Walker) on artificial diet. Journal of Chemical Ecology 27:359-370.

Carlson A (1992) Body size, prey handling efficiency and choice of diet in three coniferous forest tits (Paridae). Biological Journal of the Linnean Society 46:299-308.

Caro TM (2005) Antipredator Defenses in Birds and Mammals. University Of Chicago Press.

Caro TM, Graham CM, Stoner CJ, Vargas JK (2004) Adaptive significance of antipredator behaviour in artiodactyls. Animal Behaviour 67:205-228.

Cattin M-F, Bersier L-F, Banasek-Richter C, Baltensperger R, Gabriel J-P (2004) Phylogenetic constraints and adaptation explain food-web structure. Nature 427:835-839.

Cavender-Bares J, Kozak KH, Fine PVA, Kembel SW (2009) The merging of community ecology and phylogenetic biology. Ecology Letters 12:693-715.

Charnov EL (1976a) Optimal foraging attack strategy of a mantid. American Naturalist 110:141-151.

Charnov EL (1976b) Optimal foraging the Marginal Value Theorem. Theoretical Population Biology 9:129-136.

Charnov EL, Krebs JR (1975) The evolution of alarm calls: altruism or manipulation? American Naturalist 109:107-112.

Chau A, Mackauer M (2000) Host-instar selection in the aphid parasitoid *Monoctonus paulensis* (Hymenoptera: Braconidae, Aphidiinae): A preference for small pea aphids. European Journal of Entomology 97:347-353.

Chau A, Mackauer M (2001a) Host-instar selection in the aphid parasitoid *Monoctonus paulensis* (Hymenoptera: Braconidae, Aphidiinae): Assessing costs and benefits. The Canadian Entomologist 133:549-564.

Chau A, Mackauer M (2001b) Preference of the aphid parasitoid *Monoctonus paulensis* (Hymenoptera: Braconidae, Aphidiinae) for different aphid species: female choice and offspring survival. Biological Control 20:30-38.

Chen X, Jiang Y (2006) Diet of Chinese skink, *Eumeces chinensis*: is prey size important? Integrative Zoology 1:59-66.

Chen Y, Giles KL, Greenstone MH (2002) Molecular evidence for a species complex in the genus *Aphelinus* (Hymenoptera: Aphelinidae), with additional data on aphidiine phylogeny (Hymenoptera: Braconidae). Annals of the Entomological Society of America 95:29-34.

Cheng R-X, Meng L, Li B-P (2010) Effects of aposymbiotic and symbiotic aphids on parasitoid progeny development and adult oviposition behavior within aphid instars. Environmental Entomology 39:389-395.

Christensen B (1996) Predator foraging capabilities and prey antipredator behaviours: Pre- versus postcapture constraints on size-dependent predator-prey interactions. Oikos 76:368-380.

Clarke A, Johnston NM (1999) Scaling of metabolic rate with body mass and temperature in teleost fish. Journal of Animal Ecology 68:893-905.

Cloutier C, Duperron J, Tertuliano M, McNeil JN (2000) Host instar, body size and fitness in the koinobiotic parasitoid *Aphidius nigripes*. Entomologia Experimentalis et Applicata 97:29-40.

Cohen JE, Jonsson T, Carpenter SR (2003) Ecological community description using the food web, species abundance, and body size. Proceedings of the National Academy of Sciences of the United States of America 100:1781-1786.

Cohen JE, Jonsson T, Müller CB, Godfray HCJ, Savage VM (2005) Body sizes of hosts and parasitoids in individual feeding relationships. Proceedings of the National Academy of Sciences of the United States of America 102:684-689.

Cohen JE, Newman CM (1985) A stochastic theory of community food webs: I. Models and aggregated data. Proceedings of the Royal Society of London. Series B. Biological Sciences 224:421-448.

Cohen JE, Pimm SL, Yodzis P, Saldana J (1993) Body sizes of animal predators and animal prey in food webs. Journal of Animal Ecology 62:67-78.

Colinet H, Boivin G, Hance T (2007) Manipulation of parasitoid size using temperature-size rule: fitness consequences. Oecologia 152:425-433.

Colinet H, Salin C, Boivin G, Hance T (2005) Host age and fitness-related traits in a koinobiont aphid parasitoid. Ecological Entomology 30:473-479.

Cooper Jr WE, Anderson RA (2006) Adjusting prey handling times and methods affects profitability in the broad-headed skink (*Eumeces laticeps*). Herpetologica 62:356-365.

Cornell HV, Lawton JH (1992) Species interactions, local and regional processes, and limits to the richness of ecological communities: A theoretical perspective. Journal of Animal Ecology 61:1-12.

Costa GC, Vitt LJ, Pianka ER, Mesquita DO, Colli GR (2008) Optimal foraging constrains macroecological patterns: body size and dietary niche breadth in lizards. Global Ecology and Biogeography 17:670-677.

Cowie RJ (1977) Optimal foraging in great tits (*Parus major*). Nature 268:137-139.

Cruz-Neto AP, Andrade DV, Abe AS (2001) Energetic and physiological correlates of prey handling and ingestion in lizards and snakes. Comparative Biochemistry and Physiology A - Molecular and Integrative Physiology 128:515-533.

Damiens D, Boivin G (2006) Why do sperm-depleted parasitoid males continue to mate? Behavioral Ecology 17:138-143.

Dawson GW, Griffiths DC, Pickett JA, Smith MC, Woodcock CM (1982) Improved preparation of (E)-ß-farnesene and its activity with economically important aphids. Journal of Chemical Ecology 8:1111-1118.

Dedryver C (1987) Biologie, écologie et dynamique de population des pucerons des céréales en climat océanique, (Ph.D. thesis). University of Paris XI, Paris, France.

Desneux N, Barta RJ, Hoelmer KA, Hopper KR, Heimpel GE (2009) Multifaceted determinants of host specificity in an aphid parasitoid. Oecologia 160:387-398.

Dillwith JW, Neese PA, Brigham DL (1993) Lipid biochemistry in aphids. In: Stanley-Samuelson DW, Nelson DR (eds) Insect Lipids: chemistry, biochemistry, and biology. University of Nebraska Press, Lincoln, pp 389-434.

Dixon AFG (1958) The escape responses shown by certain aphids to the presence of the coccinellid *Adalia decempunctata* (L.). Transactions of the Royal Entomological Society of London 110:319-334.

Dixon AFG (1977) Aphid ecology: Life cycles, polymorphism, and population regulation. Annual Review of Ecology and Systematics 8:329-353.

Dixon AFG, Chambers RJ, Dharma TR (1982) Factors affecting size in aphids with particular reference to the black bean aphid, *Aphis fabae*. Entomologia Experimentalis et Applicata 32:123-128.

Dobzhansky T (1964) Biology, molecular and organismic. American Zoologist 4:443-452.

Du YJ, Poppy GM, Powell W, Pickett JA, Wadhams LJ, Woodcock CM (1998) Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. Journal of Chemical Ecology 24:1355-1368.

Dugatkin LA (1997) Cooperation among Animals: An Evolutionary Perspective. Oxford University Press, Oxford, UK.

Edwards JS (1966) Defence by smear: Supercooling in the cornicle wax of aphids. Nature 211:73-74.

Eggleton P, Gaston KJ (1990) "Parasitoid" species and assemblages: Convenient definitions or misleading compromises? Oikos 59:417-421.

Emerson BC, Gillespie RG (2008) Phylogenetic analysis of community assembly and structure over space and time. Trends in Ecology and Evolution 23:619-630.

Emlen JM (1966) The role of time and energy in food preferences. American Naturalist 100:201-208.

Enkegaard A (1994) Temperature dependent functional response of *Encarsia formosa* parasitizing the Poinsettia-strain of the cotton whitefly, *Bemisia tabaci*, on Poinsettia. Entomologia Experimentalis et Applicata 73:19-29.

Ens BJ, Esselink P, Zwarts L (1990) Kleptoparasitism as a problem of prey choice: a study on mudflat feeding curlews, *Numenius arquata*. Animal Behaviour 39:219-230.

Faria LDB, Godoy WAC, Trinca LA (2004) Dynamics of handling time and functional response by larvae of *Chrysomya albiceps* (Dipt., Calliphoridae) on different prey species. Journal of Applied Entomology 128:432-436.

Fauth JE, Bernardo J, Camara M, Resetarits WJ, Jr, Buskirk JV, McCollum SA (1996) Simplifying the jargon of community ecology: A conceptual approach. American Naturalist 147:282-286.

Fellowes MDE, van Alphen JJM, Jervis MA (2005) Foraging Behaviour. In: Jervis MA (ed) Insects as Natural Enemies: A Practical Perspective. Springer, Dordrecht, The Netherlands, pp 1-71.

Felsenstein J (1985) Phylogenies and the comparative method. American Naturalist 125:1-15.

Fievet V, Le Guigo P, Casquet J, Poinsot D, Outreman Y (2009) Living with the dead: when the body count rises, prey stick around. Behavioral Ecology 20:251-257.

Fishman MA (1999) Predator inspection: closer approach as a way to improve assessment of potential threats. Journal of Theoretical Biology 196:225-235.

Fletcher JA, Doebeli M (2009) A simple and general explanation for the evolution of altruism. Proceedings of the Royal Society B: Biological Sciences 276:13-19.

Fletcher JA, Zwick M (2004) Strong altruism can evolve in randomly formed groups. Journal of Theoretical Biology 228:303-313.

Fletcher JA, Zwick M, Doebeli M, Wilson DS (2006) What's wrong with inclusive fitness? Trends in Ecology and Evolution 21:597-598.

Forbes LS (1989) Prey defences and predator handling behaviour: The dangerous prey hypothesis. Oikos 55:155-158.

Foster KR, Wenseleers T, Ratnieks FLW (2006) Kin selection is the key to altruism. Trends in Ecology and Evolution 21:57-60.

Foster WA (2002) Soldier aphids go cuckoo. Trends in Ecology and Evolution 17:199-200.

Freckleton RP, Harvey PH, Pagel MD (2002) Phylogenetic analysis and comparative data: A test and review of evidence. American Naturalist 160:712-726.

Futuyma DJ, Moreno G (1988) The evolution of ecological specialization. Annual Review of Ecology and Systematics 19:207-233.

Gadagkar R (2009) Interrogating an insect society. Proceedings of the National Academy of Sciences of the United States of America 106:10407-10414.

Garland TJ, Bennett AF, Rezende EL (2005) Phylogenetic approaches in comparative physiology. Journal of Experimental Biology 208:3015-3035.

Gauld ID (1988) Evolutionary patterns of host utilization by ichneumonoid parasitoids (Hymenoptera: Ichneumonidae and Braconidae). Biological Journal of the Linnean Society 35:351-377.

Gerling D, Roitberg BD, Mackauer M (1990) Instar-specific defense of the pea aphid *Acyrthosiphon pisum*: Influence of oviposition success of the parasite *Aphelinus asychis* (Hymenoptera: Aphelinidae). Journal of Insect Behavior 3:501-514.

Gill AB (2003) The dynamics of prey choice in fish: the importance of prey size and satiation. Journal of Fish Biology 63:105-116.

Gill AB, Hart PJB (1994) Feeding behaviour and prey choice of the threespine stickleback: The interacting effects of prey size, fish size and stomach fullness. Animal Behaviour 47:921-932.

Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. Science 293:2248-2251.

Gillooly JF, Charnov EL, West GB, Savage VM, Brown JH (2002) Effects of size and temperature on developmental time. Nature 417:70-73.

Giraldeau L-A (2008) Solitary foraging strategies. In: Danchin É, Giraldeau L-A, Cézilly F (eds) Behavioural Ecology: An Evolutionary Perspective on Behaviour. Oxford University Press, Oxford, pp 233-256.

Giri MK, Pass BC, Yeargan KV, Parr JC (1982) Behavior, net reproduction, longevity, and mummy-stage survival of *Aphidius matricariae* [Hym. Aphidiidae]. Entomophaga 27:147-154.

Girling RD, Hassall M, Turner JG, Poppy GM (2006) Behavioural responses of the aphid parasitoid *Diaeretiella rapae* to volatiles from *Arabidopsis thaliana* induced by *Myzus persicae*. Entomologia Experimentalis et Applicata 120:1-9.

Godfray HCJ (1994) Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press, Princeton, New Jersey.

Godfray HCJ, Shimada M (1999) Parasitoids as model organisms for ecologists. Researches on Population Ecology 41:3-10.

Goss-Custard JD, West AD, Yates MG, Caldow RWG, Stillman RA, Bardsley L, Castilla J, Castro M, Dierschke V, Durell SEALVD, Eichhorn G, Ens BJ, Exo K-M, Udayangani-Fernando PU, Ferns PN, Hockey PAR, Gill JA, Johnstone I, Kalejta-Summers B, Masero JA, Moreira F, Nagarajan RV, Owens IPF, Pacheco C, Perez-

Hurtado A, Rogers D, Scheiffarth G, Sitters H, Sutherland WJ, Triplet P, Worrall DH, Zharikov Y, Zwarts L, Pettifor RA (2006) Intake rates and the functional response in shorebirds (Charadriiformes) eating macro-invertebrates. Biological Reviews 81:501-529.

Grafen A (1989) The phylogenetic regression. Philosophical Transactions of the Royal Society of London B Biological Sciences 326:119-157.

Griffiths D (1980a) The feeding biology of Ant-lion (*Morter obscurus*) larvae: Prey capture handling and utilization. Journal of Animal Ecology 49:99-126.

Griffiths D (1980b) Foraging costs and relative prey size. American Naturalist 116:743-752.

Gross P (1993) Insect behavioral and morphological defenses against parasitoids. Annual Review of Entomology 38:251-273.

Hamilton WD (1963) The evolution of altruistic behavior. American Naturalist 97:354-356.

Hamilton WD (1964) The genetical evolution of social behaviour. I. Journal of Theoretical Biology 7:1-16.

Hance T, van Baaren J, Vernon P, Boivin G (2007) Impact of extreme temperatures on parasitoids in a climate change perspective. Annual Review of Entomology 52:107-126.

Hardy ICW (1994) Sex ratio and mating structure in the parasitoid Hymenoptera. Oikos 69:3-20.

Harvey JA, Harvey IF, Thompson DJ (1994) Flexible larval growth allows use of a range of host sizes by a parasitoid wasp. Ecology 75:1420-1428.

Harvey PH (1996) Phylogenies for ecologists. Journal of Animal Ecology 65:255-263.

Harvey PH, Pagel MD (1991) The comparative method in evolutionary biology. Oxford University Press, Oxford.

Hassell MP, Southwood TRE (1978) Foraging strategies of insects. Annual Review of Ecology and Systematics 9:75-98.

He XZ, Wang Q, Teulon DAJ (2005) Host stage preference and reproductive fitness of *Aphidius eady* (Hymenoptera: Aphidiidae) on *Acyrthosiphon pisum* (Hemiptera: Aphididae). New Zealand Journal of Agricultural Research 48:157-163.

Heimpel GE, Mangel M, Rosenheim JA (1998) Effects of time limitation and egg limitation on lifetime reproductive success of a parasitoid in the field. American Naturalist 152:273-289.

Heimpel GE, Rosenheim JA (1998) Egg limitation in parasitoids: A review of the evidence and a case study. Biological Control 11:160-168.

Heinrich B (1995) Insect thermoregulation. Endeavour 19:28-33.

Henry L, Ma B, Roitberg B (2009) Size-mediated adaptive foraging: a host-selection strategy for insect parasitoids. Oecologia 161:433-445.

Henry LM, Roitberg BD, Gillespie DR (2006) Covariance of phenotypically plastic traits induces an adaptive shift in host selection behaviour.

Proceedings of the Royal Society of London Series B 273:2893-2899.

Hixon MA (1982) Energy maximizers and time minimizers: theory and reality. American Naturalist 119:596-599.

Hjelm J, Persson L (2001) Size-dependent attack rate and handling capacity: Inter-cohort competition in a zooplanktivorous fish. Oikos 95:520-532.

Hofsvang T, Hågvar EB (1975a) Duration of development and longevity in *Aphidius ervi* and *Aphidius platensis* (Hym.: Aphidiidae), two parasites of *Myzus persicae* (Hom.: Aphididae). Entomophaga 20:11-22.

Hofsvang T, Hågvar EB (1975b) Fecundity and oviposition period of *Aphidius platensis* Brèthes (Hym., Aphidiidae) parasitizing *Myzus persicae* Sulz. (Hom., Aphididae) on paprika. Norwegian Journal of Entomology 22:113-116.

Hofsvang T, Hågvar EB (1986) Oviposition behaviour of *Ephedrus cerasicola* (Hym.: Aphidiidae) parasitizing different instars of its aphid host. Entomophaga 31:261-267.

Hölldobler B, Wilson EO (1990) The Ants. Harvard University Press, Cambridge, MA.

Hollën LI, Radford AN (2009) The development of alarm call behaviour in mammals and birds. Animal Behaviour 78:791-800.

Holling CS (1959) Some characteristics of simple types of predation and parasitism. The Canadian Entomologist 91:385-398.

Howe NR, Sheikh YM (1975) Anthopleurine: a sea anemone alarm pheromone. Science 189:386-388.

Hughes RN (ed) (1993) Diet Selection: An Interdisciplinary Approach to Foraging Behaviour. Blackwell Scientific Publications, Oxford.

Ives AR, Godfray HCJ (2006) Phylogenetic analysis of trophic associations. American Naturalist 168:E1-E14.

Jablonski D, Sepkoski JJ, Jr (1996) Paleobiology, community ecology, and scales of ecological pattern. Ecology 77:1367-1378.

Jenner W, Kuhlmann U (2006) Significance of host size for a solitary endoparasitoid: A trade-off between fitness parameters. Basic and Applied Ecology 7:461-471.

Jeschke JM (2007) When carnivores are “full and lazy”. Oecologia 152:357-364.

Jeschke JM, Tollrian R (2000) Density-dependent effects of prey defences. Oecologia 123:391-396.

Johansson J, Turesson H, Persson A (2004) Active selection for large guppies, *Poecilia reticulata*, by the pike cichlid, *Crenicichla saxatilis*. Oikos 105:595-605.

Jombart T, Dray S (2008) adephylo: exploratory analyses for the phylogenetic comparative method. 1.0-2.

Jones JC, Oldroyd BP (2006) Nest thermoregulation in social insects. In: Advances in Insect Physiology, vol 33. Academic Press, pp 153-191.

Kacelnik A (1984) Central place foraging in starlings *Sturnus vulgaris* 1. Patch residence time. Journal of Animal Ecology 53:283-300.

Kairo MTK, Murphy ST (1999a) Host age choice for oviposition in *Pauesia juniperorum* (Hymenoptera: Braconidae: Aphidiinae) and its effect on the parasitoid's biology and host population growth. Biocontrol Science and Technology 9:475-486.

Kairo MTK, Murphy ST (1999b) Temperature and plant nutrient effects on the development, survival and reproduction of *Cinara* sp. nov., an invasive pest of cypress trees in Africa. Entomologia Experimentalis et Applicata 92:147-156.

Kambhampati S, Völkl W, Mackauer M (2000) Phylogenetic relationships among genera of Aphidiinae (Hymenoptera: Braconidae) based on DNA sequence of the mitochondrial 16S rRNA gene. Systematic Entomology 25:437-445.

Kavallieratos NG, Tomanović Ž, Sarlis GP, Fasseas C, Emmanouel NE (2006) A review of the genus *Aphidius* Nees in Greece (Hymenoptera : Braconidae : Aphidiinae) with the description of a new species. Journal of Natural History 40:1179-1197.

Kavallieratos NG, Tomanović Ž, Starý P, Athanassiou CG, Sarlis GP, Petrović O, Niketic M, Veroniki MA (2004) A survey of aphid parasitoids (Hymenoptera : Braconidae : Aphidiinae) of Southeastern Europe and their aphid-plant associations. Applied Entomology and Zoology 39:527-563.

Kembel S, Ackerly D, Blomberg S, Cowan P, Helmus M, Morlon H, Webb C (2009) picante: R tools for integrating phylogenies and ecology. R package version 0.7-0.

Kerr B, Godfrey-Smith P, Feldman MW (2004) What is altruism? Trends in Ecology and Evolution 19:135-140.

Kim HJ, Lee SH (2008) A molecular phylogeny of the tribe Aphidini (Insecta: Hemiptera: Aphididae) based on the mitochondrial tRNA/COII, 12S/16S and the nuclear EF1α genes. Systematic Entomology 33:711-721.

Kingsolver JG, Huey RB (2008) Size, temperature, and fitness: three rules. Evolutionary Ecology Research 10:251-268.

Kohlsdorf T, Grizante MB, Navas CA, Herrel A (2008) Head shape evolution in Tropidurinae lizards: Does locomotion constrain diet? Journal of Evolutionary Biology 21:781-790.

Korb J, Heinze J (2008) The Ecology of Social Life: A Synthesis. In: Korb J, Heinze J (eds) Ecology of Social Evolution. Springer-Verlag, Berlin, pp 245-259.

Kouamé KL, Mackauer M (1991) Influence of aphid size, age and behaviour on host choice by the parasitoid wasp *Ephedrus californicus*: a test of host-size models. Oecologia 88:197-203.

Krams I, Krama T, Igaune K (2006) Alarm calls of wintering great tits *Parus major*: warning of mate, reciprocal altruism or a message to the predator? Journal of Avian Biology 37:131-136.

Krebs JR, Davies NB (1997) Behavioural Ecology: An Evolutionary Approach, 4th edn. Blackwell Science Ltd, Oxford, UK.

Kunert G, Otto S, Röse USR, Weisser WW (2005) Alarm pheromone mediates production of winged dispersal morphs in aphids. Ecology Letters 8:596-603.

Kutsukake M, Shibao H, Uematsu K, Fukatsu T (2009) Scab formation and wound healing of plant tissue by soldier aphid. Proceedings of the Royal Society B: Biological Sciences 276:1555-1563.

Lambers DHR (1966) Polymorphism in Aphididae. Annual Review of Entomology 11:47-78.

Langer A, Boivin G, Hance T (2004) Oviposition, flight and walking capacity at low temperatures of four aphid parasitoid species (Hymenoptera : Aphidiinae). European Journal of Entomology 101:473-479.

Latta B (1987) Adaptive and non-adaptive suicide in aphids. Nature 330:701.

Lawton JH (1999) Are there general laws in ecology? Oikos 84:177-192.

Lawton JH, Warren PH (1988) Static and dynamic explanations for patterns in food webs. Trends in Ecology and Evolution 3:242-245.

Layman CA, Winemiller KO, Arrington DA, Jepsen DB (2005) Body size and trophic position in a diverse tropical food web. Ecology 86:2530-2535.

Leibold MA, Holyoak M, Mouquet N, Amarasekare P, Chase JM, Hoopes MF, Holt RD, Shurin JB, Law R, Tilman D, Loreau M, Gonzalez A (2004) The metacommunity concept: a framework for multi-scale community ecology. Ecology Letters 7:601-613.

Lemon WC (1991) Fitness consequences of foraging behavior in the zebra finch. Nature 352:153-155.

Lemon WC, Barth RHJ (1992) The effects of feeding rate on reproductive success in the zebra finch, *Taeniopygia guttata*. Animal Behaviour 44:851-857.

Lester PJ, Holtzer TO (2002) Patch and prey utilization behaviors by *Aphelinus albipodus* and *Diaeretiella rapae* (Hymenoptera: Aphelinidae and Aphidiidae) on russian wheat aphid (Homoptera: Aphididae). Biological Control 24:183-191.

Liu S-S (1985) Development, adult size and fecondity of *Aphidius sonchi* reared in two instars of its aphid host, *Hyperomyzus lactucae*. Entomologia Experimentalis et Applicata 37:41-48.

Liu Y, Hu C, Ni HX, Sun JR (2001) Effects of volatiles from different trophic level on foraging behavior of *Aphidius avenae*. Yingyong Shengtai Xuebao 12:581-584.

Losos JB (1996) Phylogenies and comparative biology, stage II: Testing causal hypotheses derived from phylogenies with data from extant taxa. Systematic Biology 45:259-260.

Losos JB (2008) Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecology Letters 11:995-1003.

Loxdale HD (2008) The nature and reality of the aphid clone: genetic variation, adaptation and evolution. Agricultural and Forest Entomology 10:81-90.

Lucas E, Brodeur J (2001) A fox in sheep's clothing: furtive predators benefit from the communal defense of their prey. Ecology 82:3246-3250.

Lykouressis D, Garantonakis N, Perdikis D, Fantinou A, Mauromoustakos A (2009) Effect of female size on host selection by a koinobiont insect parasitoid (Hymenoptera: Braconidae: Aphidiinae). European Journal of Entomology 106:363-367.

MacArthur RH, Pianka ER (1966) On optimal use of a patchy environment. American Naturalist 100:603-609.

Mackauer M (1983) Quantitative assessment of *Aphidius smithi* (Hymenoptera, Aphidiidae) fecundity, intrinsic rate of increase and functional response. The Canadian Entomologist 115:399-416.

Mackauer M, Sequeira R (1993) Patterns of development in insect parasites. In: Beckage NE, Thompson SN, Federici BA (eds) Parasites and Pathogens of Insects, vol 1. Academic Press, San Diego, CA, pp 1-23.

Martel V, Wajnberg E, Boivin G (2008) Patch time allocation in male parasitoids. Ecological Entomology 33:608-613.

Martinez ND, Lawton JH (1995) Scale and food-web structure: From local to global. Oikos 73:148-154.

Martins EP, Diniz Filho JAF, Housworth EA (2002) Adaptive constraints and the phylogenetic comparative method: A computer simulation test. Evolution 56:1-13.

Maynard-Smith J (1964) Group selection and kin selection. Nature 201:1145-1147.

McAllister MK, Roitberg BD (1987) Adaptive suicidal behaviour in pea aphids. Nature 328:797-799.

McAllister MK, Roitberg BD, Weldon KL (1990) Adaptive suicide in pea aphids: Decisions are cost sensitive. Animal Behaviour 40:167-175.

Medvedev GS (1995) Keys to the Insects of the European Part of the USSR. Institute of Zoology, Academy of Sciences of the USSR.

Meehl GA, Stocker TF, Collins WD, Friedlingstein P, Gaye AT, Gregory JM, Kitoh A, Knutti R, Murphy JM, Noda A, Raper SCB, Watterson IG, Weaver AJ, Zhao Z-C (2007) Global Climate Projections. In: Solomon S et al. (eds) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, U.S.A., pp 747-846.

Miao XX, Huang YP, Zhu XX, Ding D (2004) A comparative study on development and reproduction of the parasitoid *Lysiphlebus japonicus* (Hymenoptera: Aphidiidae) in symbiotic and aposymbiotic host aphids. Applied Entomology and Zoology 39:243-248.

Micha SG, Wyss U (1996) Aphid alarm pheromone (E)-ß-farnesene: a host finding kairomone for the aphid primary parasitoid *Aphidius uzbekistanicus* (Hymenoptera: Aphidiinae). Chemoecology 7:132-139.

Michod RE (1982) The theory of kin selection. Annual Review of Ecology and Systematics 13:23-55.

Miles DB, Dunham AE (1993) Historical perspectives in ecology and evolutionary biology: The use of phylogenetic comparative analyses. Annual Review of Ecology and Systematics 24:587-619.

Millor J, Pham-Delegue M, Deneubourg JL, Camazine S (1999) Self-organized defensive behavior in honeybees. Proceedings of the National Academy of Sciences of the United States of America 96:12611-12615.

Minkenberg OPJM, Tatar M, Rosenheim JA (1992) Egg load as a major source of variability in insect foraging and oviposition behavior. Oikos 65:134-142.

Mondor EB, Roitberg BD (2000) Has the attraction of predatory coccinellids to cornicle droplets constrained aphid alarm signaling behavior? Journal of Insect Behavior 13:321-329.

Mondor EB, Roitberg BD (2003) Age dependent fitness costs of alarm signaling in aphids. Canadian Journal of Zoology 81:757-762.

Mondor EB, Roitberg BD (2004) Inclusive fitness benefits of scent-marking predators. Proceedings of the Royal Society B: Biological Sciences 271:S341-S343.

Mondor EB, Roitberg BD, Stadler B (2002) Cornicle length in Macrosiphini aphids: A comparison of ecological traits. Ecological Entomology 27:758-762.

Montgomerie RD, Weatherhead PJ (1988) Risks and rewards of nest defence by parent birds. Quarterly Review of Biology 63:167-187.

Montgomery ME, Nault LR (1977) Comparative response of aphids to the alarm pheromone E-ß-farnesene. Entomologia Experimentalis et Applicata 22:236-242.

Morse DH, Stephens EG (1996) The consequences of adult foraging success on the components of lifetime fitness in a semelparous, sit and wait predator. Evolutionary Ecology 10:361-373.

Müller CB, Williams IS, Hardie J (2001) The role of nutrition, crowding and interspecific interactions in the development of winged aphids. Ecological Entomology 26:330-340.

Muratori F, Damiens D, Hance T, Boivin G (2008) Bad housekeeping: why do aphids leave their exuviae inside the colony? BMC Evolutionary Biology 8:338.

Nespolo RF, Lardies MA, Bozinovic F (2003) Intrapopulational variation in the standard metabolic rate of insects: repeatability, thermal dependence and sensitivity (Q10) of oxygen consumption in a cricket. Journal of Experimental Biology 206:4309-4315.

Neubert MG, Blumenshine SC, Duplisea DE, Jonsson T, Rashleigh B (2000) Body size and food web structure: testing the equiprobability assumption of the cascade model. Oecologia 123:241-251.

Nicol CMY, Mackauer M (1999) The scaling of body size and mass in a host parasitoid association: influence of host species and stage. Entomologia Experimentalis et Applicata 90:83-92.

Normark BB (2000) Molecular systematics and evolution of the aphid family Lachnidae. Molecular Phylogenetics and Evolution 14:131-140.

Ode PJ, Hopper KR, Coll M (2005) Oviposition vs. offspring fitness in *Aphidius colemani* parasitizing different aphid species. Entomologia Experimentalis et Applicata 115:303-310.

Oliver KM, Russell JA, Moran NA, Hunter MS (2003) Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. Proceedings of the National Academy of Sciences of the United States of America 100:1803-1807.

Ortiz-Rivas B, Moya A, Martínez-Torres D (2004) Molecular systematics of aphids (Homoptera : Aphididae): new insights from the long-wavelength opsin gene. Molecular Phylogenetics and Evolution 30:24-37.

Outreman Y, Kunert G, Simon J-C, Weisser WW (2010) Ecological costs of alarm signalling in aphids. In: Kindlmann P, Dixon AFG, Michaud JP (eds) Aphid Biodiversity Under Environmental Change: Patterns and Processes. Springer, Dordrecht, pp 171-181.

Outreman Y, Le Ralec A, Plantegenest M, Chaubet B, Pierre J-S (2001) Superparasitism limitation in an aphid parasitoid: cornicle secretion avoidance and host discrimination ability. Journal of Insect Physiology 47:339-348.

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.

Pan W (2001) Akaike's information criterion in Generalized Estimating Equations. Biometrics 57:120-125.

Paradis E (2006) Analysis of Phylogenetics and Evolution with R. Springer, New York, NY.

Paradis E, Claude J, Strimmer K (2004) APE: Analyses of Phylogenetics and Evolution in R language. Bioinformatics 20:289-290.

Pasteels JM, Grégoire JC, Rowell-Rahier M (1983) The chemical ecology of defense in arthropods. Annual Review of Entomology 28:263-289.

Pavoine S, Ollier S, Pontier D, Chessel D (2008) Testing for phylogenetic signal in phenotypic traits: New matrices of phylogenetic proximities. Theoretical Population Biology 73:79-91.

Persad AB, Hoy MA (2003) Intra and interspecific interactions between *Lysiphlebus testaceipes* and *Lipolexis scutellaris* (Hymenoptera:

Aphidiidae) reared on *Toxoptera citricida* (Homoptera: Aphididae). Journal of Economic Entomology 96:564-569.

Petchey OL, Beckerman AP, Riede JO, Warren PH (2008) Size, foraging, and food web structure. Proceedings of the National Academy of Sciences of the United States of America 105:4191-4196.

Peters RH (1983) The Ecological Implications of Body Size. Cambridge University Press, Cambridge.

Pfennig DW (2000) Effect of predator-prey phylogenetic similarity on the fitness consequences of predation: A trade-off between nutrition and disease? American Naturalist 155:335-345.

Pickett JA, Griffiths DC (1980) Composition of aphid alarm pheromones. Journal of Chemical Ecology 6:349-360.

Pickett JA, Wadhams LJ, Woodcock CM, Hardie J (1992) The chemical ecology of aphids. Annual Review of Entomology 37:67-90.

Pierre J-S, van Baaren J, Boivin G (2003) Patch leaving decision rules in parasitoids: do they use sequential decisional sampling? Behavioral Ecology and Sociobiology 54:147-155.

Pike KS, Starý P (1996) New species of *Pauesia* (Hymenoptera: Braconidae: Aphidiinae) parasitoids of *Cinara* (Homoptera: Aphididae: Lachninae) associated with conifers in the Pacific Northwest. Proceedings of the Entomological Society of Washington 98:324-331.

Pike N, Foster WA (2008) The Ecology of Altruism in a Clonal Insect. In: Korb J, Heinze J (eds) Ecology of Social Evolution. Springer-Verlag, Berlin, pp 37-56.

Pinheiro J, Bates D, DebRoy S, Sarkar D, team RC (2009) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-92.

Pitcher T (1992) Who dares wins: the function and evolution of predator inspection behaviour in shoaling fish. Netherlands Journal of Zoology 42:371-391.

Prestwich GD (1984) Defense mechanisms of termites. Annual Review of Entomology 29:201-232.

Price T (1997) Correlated evolution and independent contrasts. Philosophical Transactions of the Royal Society of London B Biological Sciences 352:519-529.

Pusey AE (2005) Social systems. In: Bolhuis JJ, Giraldeau L-A (eds) The Behavior of Animals: Mechanisms, function, and evolution. Blackwell Publishing, Malden, MA, pp 315-341.

Pyke GH, Pulliam HR, Charnov EL (1977) Optimal foraging: A selective review of theory and tests. Quarterly Review of Biology 52:137-154.

Qayyum A (2001) Effects of host age on two closely related parasitoid species *Diaeretiella rapae* (mcintosh) and *Aphidius colemani* (Viereck) (Aphidiidae: Hymenoptera). Pakistan Journal of Zoology 33:193-200.

Queller DC, Strassmann JE (2003) Eusociality. Current Biology 13:R861-R863.

R Development Core Team (2009) R: A language and environment for statistical computing.

Radloff FGT, Du Toit JT (2004) Large predators and their prey in a southern African savanna: A predator's size determines its prey size range. Journal of Animal Ecology 73:410-423.

Rao TR, Kumar R (2002) Patterns of prey selectivity in the cyclopoid copepod *Mesocyclops thermocyclopoides*. Aquatic Ecology 36:411-424.

Ratnieks FLW, Wenseleers T (2008) Altruism in insect societies and beyond: voluntary or enforced? Trends in Ecology and Evolution 23:45-52.

Reuter OM (1913) Lebensgewohnheiten und Instinkte der Insekten. Friedlander, Berlin.

Rezende EL, Albert EM, Fortuna MA, Bascompte J (2009) Compartments in a marine food web associated with phylogeny, body mass, and habitat structure. Ecology Letters 12:779-788.

Ricklefs RE (1987) Community diversity: Relative roles of local and regional processes. Science 235:167-171.

Ricklefs RE (2008) Disintegration of the ecological community. American Naturalist 172:741-750.

Roitberg BD, Boivin G, Vet LEM (2001) Fitness, parasitoids, and biological control: an opinion. The Canadian Entomologist 133:429-438.

Rose TA, Munn AJ, Ramp D, Banks PB (2006) Foot-thumping as an alarm signal in macropodoid marsupials: prevalence and hypotheses of function. Mammal Review 36:281-298.

Rosenheim JA (1999) The relative contributions of time and eggs to the cost of reproduction. Evolution 53:376-385.

Rosenheim JA, Jepsen SJ, Matthews CE, Smith DS, Rosenheim MR (2008) Time limitation, egg limitation, the cost of oviposition, and lifetime reproduction by an insect in nature. American Naturalist 172:486-496.

Rotheray GE, Barbosa P (1984) Host related factors affecting oviposition behavior in *Brachymeria intermedia*. Entomologia Experimentalis et Applicata 35:141-146.

Rovero F, Hughes RN, Chelazzi G (2000) When time is of the essence: Choosing a currency for prey-handling costs. Journal of Animal Ecology 69:683-689.

Rueffler C, Van Dooren TJM, Leimar O, Abrams PA (2006) Disruptive selection and then what? Trends in Ecology and Evolution 21:238-245.

Ryan MJ (2005) The evolution of behaviour, and integrating it towards a complete and correct understanding of behavioural biology. Animal Biology 55:419-439.

Sabater B, van Ham RCHJ, Martínez-Torres D, Silva F, Latorre A, Moya A (2001) Molecular evolution of aphids and their primary (*Buchnera* sp.) and secondary endosymbionts: Implications for the role of symbiosis in insect evolution. INCI 26:508-512.

Sampaio MV, Bueno VHP, Rodrigues SMM, Soglia MdCdM, De Conti BF (2007) Desenvolvimento de *Aphidius colemani* Viereck (Hymenoptera: Braconidae, Aphidiinae) e alterações causadas pelo parasitismo no hospedeiro *Aphis gossypii* Glover (Hemiptera: Aphididae) em diferentes temperaturas. Neotropical Entomology 36:436-444.

Sanchis A, Michelena JM, Latorre A, Quicke DLJ, Gardenfors U, Belshaw R (2001) The phylogenetic analysis of variable-length sequence data: Elongation factor-1α introns in European populations of the parasitoid wasp genus *Pauesia* (Hymenoptera: Braconidae: Aphidiinae). Molecular Biology and Evolution 18:1117-1131.

Sato A, O'hUigin C, Figueroa F, Grant PR, Grant BR, Tichy H, Klein J (1999) Phylogeny of Darwin's finches as revealed by mtDNA sequences. Proceedings of the National Academy of Sciences of the United States of America 96:5101-5106.

Schoener TW (1971) Theory of feeding strategies. Annual Review of Ecology and Systematics 2:369-404.

Schreiber SJ, Vejdani M (2006) Handling time promotes the coevolution of aggregation in predator-prey systems. Proceedings of the Royal Society Biological Sciences Series B 273:185-191.

Searcy WA, Nowicki S (2005) The Evolution of Animal Communication: Reliability and Deception in Signaling systems. Princeton University Press, Princeton, NJ.

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

Shavit A, Millstein RL (2008) Group selection is dead! Long live group selection? BioScience 58:574-575.

Shelley EL, Blumstein DT (2005) The evolution of vocal alarm communication in rodents. Behavioral Ecology 16:169-177.

Shirota Y, Carter N, Rabbinge R, Ankersmit GW (1983) Biology of *Aphidius rhopalosiphi*, a parasitoid of cereal aphids. Entomologia Experimentalis et Applicata 34:27-34.

Sih A, Christensen B (2001) Optimal diet theory: When does it work, and when and why does it fail? Animal Behaviour 61:379-390.

Silva RJ, Bueno VHP, Sampaio MV (2008a) Qualidade de diferentes espécies de pulgões como hospedeiros do parasitóide *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae, Aphidiinae). Neotropical Entomology 37:173-179.

Silva RJ, Bueno VHP, Silva DB, Sampaio MV (2008b) Tabela de vida de fertilidade de *Lysiphlebus testaceipes* (Cresson) (Hymenoptera, Braconidae, Aphidiinae) em *Rhopalosiphum maidis* (Fitch) e *Aphis gossypii* Glover (Hemiptera, Aphididae). Revista Brasileira De Entomologia 52:124-130.

Slagsvold T, Sonerud GA (2007) Prey size and ingestion rate in raptors: importance for sex roles and reversed sexual size dimorphism. Journal of Avian Biology 38:650-661.

Smith CA, Sardeshmukh PD (2000) The effect of ENSO on the intraseasonal variance of surface temperatures in winter. International Journal of Climatology 20:1543-1557.

Smith PT, Kambhampati S, Völkl W, Mackauer M (1999) A phylogeny of aphid parasitoids (Hymenoptera : Braconidae : Aphidiinae) inferred from mitochondrial NADH 1 dehydrogenase gene sequence. Molecular Phylogenetics and Evolution 11:236-245.

Smith RJF (1992) Alarm signals in fishes. Reviews in Fish Biology and Fisheries 2:33-63.

Stadler B, Mackauer M (1996) Influence of plant quality on interactions between the aphid parasitoid *Ephedrus californicus* Baker (Hymenoptera: Aphidiidae) and its host, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae). The Canadian Entomologist 128:27-39.

Starý P (1964) Food specificity in the Aphidiidae (Hymenoptera). Entomophaga 9:91-99.

Starý P (1975) *Aphidius colemani* Viereck: its taxonomy, distribution and host range (Hymenoptera, Aphidiidae). Acta Entomologica Bohemoslovaca 72:156-163.

Starý P (1988) Aphidiidae. In: Minks AK, Harrewijn P (eds) Aphids: Their Biology, Natural Enemies and Control, vol B, Elsevier edn, New York, pp 171-184.

Starý P, Zuparko RL (1995) A new species of *Trioxys* (Hymenoptera: Braconidae) from California. Pan-Pacific Entomologist 71:173-175.

Steidle JLM, van Loon JJA (2003) Dietary specialization and infochemical use in carnivorous arthropods: testing a concept. Entomologia Experimentalis et Applicata 108:133-148.

Stephens DW (1981) The logic of risk sensitive foraging preferences. Animal Behaviour 29:628-629.

Stephens DW (1990) Foraging theory: Up, down, and sideways. In: Morrison ML, Ralph CJ, Verner J, Jehl JR, Jr (eds) Studies in Avian Biology, vol 13. Cooper Ornithological Society, pp 444-454.

Stephens DW, Brown JS, Ydenberg RC (eds) (2007) Foraging: Behavior and Ecology. The University of Chicago Press, Chicago.

Stephens DW, Krebs JR (1986) Foraging Theory. Princeton University Press, Princeton, NJ.

Stern DL, Foster WA (1996) The evolution of soldiers in aphids. Biological Reviews 71:27-79.

Stern DL, Moon A, Martinez del Rio C (1996) Caste allometries in the soldier-producing aphid *Pseudoregma alexanderi* (Hormaphididae: Aphidoidea). Insectes Sociaux 43:137-147.

Stevenson RD, Peterson CR, Tsuji JS (1985) The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. Physiological Zoology 58:46-57.

Stouffer DB (2010) Scaling from individuals to networks in food webs. Functional Ecology 24:44-51.

Sullivan DJ (1987) Insect Hyperparasitism. Annual Review of Entomology 32:49-70.

Swenson NG, Enquist BJ, Pither J, Thompson J, Zimmerman JK (2006) The problem and promise of scale dependency in community phylogenetics. Ecology 87:2418-2424.

Tessman I (1995) Human altruism as a courtship display. Oikos 74:157-158.

Thompson RM, Townsend CR (2005) Food-web topology varies with spatial scale in a patchy environment. Ecology 86:1916-1925.

Tofilski A, Couvillon MJ, Evison SEF, Helanterä H, Robinson EJH, Ratnieks FLW (2008) Preemptive defensive self-sacrifice by ant workers. American Naturalist 172

Tomanović Ž, Kavallieratos NG (2002) Two new aphidiine wasps from the southeastern Europe (Insecta: Hymenoptera: Braconidae: Aphidiinae). Reichenbachia Staatliches Museum Für Tierkunde Dresden 34:341-345.

Tomlinson I (1987) Adaptive and non-adaptive suicide in aphids. Nature 330:701.

Torres AdF, Bueno VHP, Sampaio MV, De Conti BF (2007) Tabela de vida de fertilidade de *Aphidius colemani* Viereck (Hymenoptera: Braconidae, Aphidiinae) em *Aphis gossypii* Glover (Hemiptera: Aphididae). Neotropical Entomology 36:532-536.

Troost T, Kooi B, Dieckmann U (2008) Joint evolution of predator body size and prey-size preference. Evolutionary Ecology 22:771-799.

Turesson H, Persson A, Bronmark C (2002) Prey size selection in piscivorous pikeperch (*Stizostedion lucioperca*) includes active prey choice. Ecology of Freshwater Fish 11:223-233.

Valone TJ (1992) Patch estimation via memory windows and the effect of travel time. Journal of Theoretical Biology 157:243-251.

van Alphen JJM, Visser ME (1990) Superparasitism as an adaptive strategy for insect parasitoids. Annual Review of Entomology 35:59-79.

van Baaren J, Hétérier V, Hance T, Krespi L, Cortesero A-M, Poinsot D, Le Ralec A, Outreman Y (2004) Playing the hare or the tortoise in parasitoids: could different oviposition strategies have an influence in host partitioning in two *Aphidius* species? Ethology Ecology and Evolution 16:231-242.

van der Have TM, de Jong G (1996) Adult size in ectotherms: Temperature effects on growth and differentiation. Journal of Theoretical Biology 183:329-340.

van Gils JA, Schenk IW, Bos O, Piersma T (2003) Incompletely informed shorebirds that face a digestive constraint maximize net energy gain when exploiting patches. American Naturalist 161:777-793.

van Lenteren JC (2006) Internet Book of Biological Control, Version 4 edn. IOBC Global, Wageningen, The Netherlands.

van Lenteren JC, Bakker K (1975) Discrimination between parasitised and unparasitised hosts in the parasitic wasp *Pseudeucoila bochei*: a matter of learning. Nature 254:417-419.

van Savage M, Gillooly James F, Brown James H, West Geoffrey B, Charnov Eric L (2004) Effects of body size and temperature on population growth. American Naturalist 163:429-441.

van Veen FJF, Memmott J, Godfray HCJ (2006) Indirect effects, apparent competition and biological control. In: Brodeur J, Boivin G (eds) Trophic and Guild Interactions in Biological Control, vol 3. Springer, Dordrecht, The Netherlands, pp 145-169.

Vellend M (2010) Conceptual synthesis in community ecology. The Quarterly Review of Biology 85:183-206.

Verheggen FJ, Haubruge E, De Moraes CM, Mescher MC (2009) Social environment influences aphid production of alarm pheromone. Behavioral Ecology 20:283-288.

Vermaat JE, Dunne JA, Gilbert AJ (2009) Major dimensions in food-web structure properties. Ecology 90:278-282.

Vet LEM (2001) Parasitoid searching efficiency links behaviour to population processes. Applied Entomology and Zoology 36:399-408.

Vet LEM, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. Annual Review of Entomology:141-172.

Vet LEM, Lewis WJ, Cardé RT (1995) Parasitoid foraging and learning. In: Bell WJ, Cardé RT (eds) Chemical Ecology of Insects, 2nd edn. Chapman and Hall, pp 65-101.

Villagra CA, Ramirez CC, Niemeyer HM (2002) Antipredator responses of aphids to parasitoids change as a function of aphid physiological state. Animal Behaviour 64:677-683.

Vincent SE, Mori A (2008) Determinants of feeding performance in free-ranging pit-vipers (Viperidae: *Ovophis okinavensis*): key roles for head size and body temperature. Biological Journal of the Linnean Society 93:53-62.

Vinson SB (1976) Host selection by insect parasitoids. Annual Review of Entomology 21:109-133.

Vinson SB, Iwantsch GF (1980) Host suitability for insect parasitoids. Annual Review of Entomology 25:397-419.

Vitt LJ, Pianka ER (2005) Deep history impacts present-day ecology and biodiversity. Proceedings of the National Academy of Sciences of the United States of America 102:7877-7881.

Vogt JT, Smith WA, Grantham RA, Wright RE (2003) Effects of temperature and season on foraging activity of red imported fire ants (Hymenoptera: Formicidae) in Oklahoma. Environmental Entomology 32:447-451.

Völkl W, Mackauer M (2000) Oviposition behaviour of aphidiine wasps (Hymenoptera: Braconidae, Aphidiinae): morphological adaptations and evolutionary trends. The Canadian Entomologist 132:197-212.

von Dohlen CD, Rowe CA, Heie OE (2006) A test of morphological hypotheses for tribal and subtribal relationships of Aphidinae (Insecta : Hemiptera : Aphididae) using DNA sequences. Molecular Phylogenetics and Evolution 38:316-329.

Vos M, Hemerik L (2003) Linking foraging behavior to lifetime reproductive success for an insect parasitoid: adaptation to host distributions. Behavioral Ecology 14:236-245.

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, Fauvergue X, Pons O (2000) Patch leaving decision rules and the marginal value theorem: an experimental analysis and a simulation model. Behavioral Ecology 11:577-586.

Walker AM, Hoy MA (2003) Responses of *Lipolexis oregmae* (Hymenoptera: Aphidiidae) to different instars of *Toxoptera citricida* (Homoptera: Aphididae). Journal of Economic Entomology 96:1685-1692.

Walters RJ, Hassall M (2006) The temperature-size rule in ectotherms: May a general explanation exist after all? American Naturalist 167:510-523.

Warren PH, Lawton JH (1987) Invertebrate predator-prey body size relationships: An explanation for upper triangular food webs and patterns in food web structure. Oecologia 74:231-235.

Webb CO, Ackerly DD, McPeek MA, Donoghue MJ (2002) Phylogenies and community ecology. Annual Review of Ecology and Systematics 33:475-505.

Webb CO, Losos JB, Agrawal AA (2006) Integrating phylogenies into community ecology. Ecology 87:1-2.

Wei JN, Bai BB, Yin TS, Wang Y, Yang Y, Zhao LH, Kuang RP, Xiang RJ (2005) Development and use of parasitoids (Hymenoptera: Aphidiidae & Aphelinidae) for biological control of aphids in China. Biocontrol Science and Technology 15:533-551.

Weiblen GD, Webb CO, Novotny V, Basset Y, Miller SE (2006) Phylogenetic dispersion of host use in a tropical insect herbivore community. Ecology 87:S62-S75.

Weisser WW (1994) Age-dependent foraging behaviour and host-instar preference of the aphid parasitoid *Lysiphlebus cardui*. Entomologia Experimentalis et Applicata 70:1-10.

Weisser WW, Völkl W, Hassell MP (1997) The importance of adverse weather conditions for behaviour and population ecology of an aphid parasitoid. Journal of Animal Ecology 66:386-400.

West SA, Gardner A, Griffin AS (2006) Altruism. Current Biology 16:R482-R483.

West SA, Griffin AS, Gardner A (2007a) Evolutionary explanations for cooperation. Current Biology 17:R661-R672.

West SA, Griffin AS, Gardner A (2007b) Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. Journal of Evolutionary Biology 20:415-432.

West SA, Murray MG, Machado CA, Griffin AS, Herre EA (2001) Testing Hamilton's rule with competition between relatives. Nature 409:510-513.

West SA, Pen I, Griffin AS (2002) Cooperation and competition between relatives. Science 296:72-75.

Wheeler BC (2008) Selfish or altruistic? An analysis of alarm call function in wild capuchin monkeys, *Cebus apella nigritus*. Animal Behaviour 76:1465-1475.

Wiens JJ, Graham CH (2005) Niche conservatism: Integrating evolution, ecology, and conservation biology. Annual Review of Ecology, Evolution, and Systematics 36:519-539.

Wientjens WHJM, Lakwijk AC, van der Marel T (1973) Alarm pheromone of grain aphids. Cellular and Molecular Life Sciences 29:658-660.

Williams RJ, Brose U, Martinez ND (2007) Homage to Yodzis and Innes 1992: Scaling up Feeding-Based Population Dynamics to Complex Ecological Networks. In: Rooney N, McCann KS, Noakes DLG (eds) From Energetics to Ecosystems: The Dynamics and Structure of Ecological Systems. Springer Netherlands, Dordrecht, The Netherlands, pp 37-51.

Williams RJ, Martinez ND (2000) Simple rules yield complex food webs. Nature 404:180-183.

Wilson DS (1990) Weak altruism, strong group selection. Oikos 59:135-140.

Wilson EO (1976) The organization of colony defense in the ant *Pheidole dentata* Mayr (Hymenoptera: Formicidae). Behavioral Ecology and Sociobiology 1:63-81.

Wilson EO (2005) Kin selection as the key to altruism: Its rise and fall. Social Research 72:159-166.

Woodland DJ, Jaafar Z, Knight M-L (1980) The "pursuit deterrent" function of alarm signals. American Naturalist 115:748-753.

Woodward G, Ebenman B, Ernmerson M, Montoya JM, Olesen JM, Valido A, Warren PH (2005) Body size in ecological networks. Trends in Ecology and Evolution 20:402-409.

Wool D (2004) Galling aphids: Specialization, biological complexity, and variation. Annual Review of Entomology 49:175-192.

Wu G-M, Barrette M, Boivin G, Brodeur J, Giraldeau L-A, Hance T (2011) Temperature affects handling time through body size-mediated effects. Environmental Entomology 40:in press.

Wu G-M, Boivin G, Brodeur J, Giraldeau L-A, Outreman Y (2010) Altruistic defence behaviours in aphids. BMC Evolutionary Biology 10:19.

XiangYu JG, Zhang F, Fang YL, Kan W, Zhang GX, Zhang ZN (2002) Behavioural response of aphids to the alarm pheromone component (*E*)-ß-farnesene in the field. Physiological Entomology 27:307-311.

Yan J (2002) geepack: Yet another package for generalized estimating equations. R-News 2/3:12-14.

Yan J, Fine J (2004) Estimating equations for association structures. Statistics in Medicine 23:859-880.

Ydenberg RC, Welham CVJ, Schmid-Hempel R, Schmid-Hempel P, Beauchamp G (1994) Time and energy constraints and the relationships between currencies in foraging theory. Behavioral Ecology 5:28-34.

Ydenberg RY, Brown JS, Stephens DW (2007) Foraging: An overview. In: Stephens DW, Brown JS, Ydenberg RC (eds) Foraging: Behavior and Ecology. Chicago University Press, Chicago.

Zamani AA, Talebi AA, Fathipour Y, Baniameri V (2006) Temperature-dependent functional response of two aphid parasitoids, *Aphidius colemani* and *Aphidius matricariae* (Hymenoptera: Aphidiidae), on the cotton aphid. Journal of Pest Science 79:183-188.

Zeger SL, Liang K-Y (1986) Longitudinal data analysis for discrete and continuous outcomes. Biometrics 42:121-130.