Inbreeding avoidance mechanism: how premating conditions may influence the mating system in a quasi-gregarious parasitoid of aphids, *Aphidius matricariae.*

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Une page se tourne, mais je n'en doute pas, de nouvelles opportunités arrivent et n'attendent qu'une chose : que je les saisissent au vol !

Abstract

The theory of sexual selection was formulated by Darwin (1871) to explain the origin of sexually dimorphic traits that are detrimental to survival. He believed that sexual selection arises from either mate choice or intra-sexual competition for mates, reasoning that such traits could evolve if they conferred a fitness advantage on their bearer. It is well conceded that males and females do not mate randomly and that mate choice is an important aspect of mating systems. Mate choice can operate on numerous traits but inbreeding avoidance is one of the major aspects that drive mate choice, from plants to society-living animals. Indeed, many species suffer from inbreeding depression and had selected inbreeding avoidance strategies: dispersal, favouring extra-pair or extra-group copulation, recognition and avoidance of kin as mate, delayed maturation, role of the social system, etc.

However optimal mate choice can also be constrained by mate availability and environmental or physiological factors. Little data are currently available on the different steps preceding the mating itself for males and females such as the place of emergence of both sex at adulthood, the probability to meet a kin in its immediate environment and how these factors may influence the final choice. It is particularly the case for Hymenoptera parasitoids, where adult emergence is constrained by the host behaviour.

Parasitoids are organisms that parasite others and kill them as a result of their development. Hymenoptera parasitoids are haplo-diploid organisms and for some groups, like aphid parasitoids (Aphidiinae: Braconidae), when a female mate with a related male, the single-locus Complementary Sex Determination mechanism may lead to the production of unfertile diploid males. Moreover, in the case of aphid parasitoids, as their host lives in groups, when a female laid its eggs in an aphid colony, a synchronous emergence of its offspring may results in Local Mate Competition. Thus, aphid parasitoids have probably developed some mechanisms to avoid mating between siblings or competition between brothers. For the Aphidiinae subfamily few studies address the consequences of inbreeding and their actual status of semi-gegarious species.

In that context, our hypothesis is first that aphid behaviour after parasitism may influence the parasitoid mating structure, secondly that aphid parasitoids have developed adapatations to avoid inbreeding and Local Mate Competition.

We show that the peach potato aphid *Myzus persicae* disperses from its colony when disturbed by a parasitoid female *Aphidius matricariae.* This induces the solitary emergence of the offspring (paper 1). We then observe

that the spatio-temporal emergence pattern of *Aphidius matricariae* adults could lower the probability to encounter a sib. Indeed, we demonstrate the existence of protandry and that the distribution of males and females emergence from a single brood spreads over 3 days (paper 2). Brothers and sisters do not often emerge at the same time, increasing the probability of outbreeding (paper 2).

Then, our second objective was to evaluate what were the traits related to mating that could favour outbreeding. The hypothesis of a differential mating window is validated: females need around 30 minutes to be receptive to males and accept to mate until they are 6 days old (paper 4). Males are sexually mature just after emergence and are capable to court and mate female during 11 days in lab conditions (paper 4). Contrary to our predictions, females usually mate only once (paper 3) and no behavioural avoidance of mating between brothers and sisters (paper 5) occur. This is probably due to the previous traits that decreased the sib mating probabilities enough to have a low rate of inbreeding in natural populations.

To conclude, *A. matricariae* exhibit different life-history strategies and mating behaviours that result in a decrease of the inbreeding probability in lab conditions. Field studies and precise sex ratio evaluations are needed to confirm and refine our observations.

Résumé

La théorie de la sélection sexuelle a été formulée par Darwin (1871) pour expliquer la sélection de certains traits qui semblent au premier abord préjudiciables à la survie de l'individu qui les exprime. Il pensait que la sélection sexuelle était sous l'influence du choix du partenaire ou d'une compétition intra-sexuelle pour l'optention du-dit partenaire. Ainsi, chaque trait devait évoluer et être sélectionné sous réserve de conférer un avantage à leur propriétaire en terme de gain de valeur adaptative (=fitness).

Il est maintenant bien admis que les mâles et les femelles ne s'accouplent pas au hasard et que le choix du partenaire est un aspect important des systèmes d'appariement. Le choix du partenaire peut se faire sur la base de nombreuses caractéristiques mais l'évitement de la consanguinité est l'un des aspects majeurs qui dirigent ce choix chez les deux sexes. En effet, beaucoup d'espèces (aussi bien animales que végétales) souffrent des effets de la consanguinité, si bien que des mécanismes d'évitement d'un accouplement consanguin ont été sélectionés: dispersion, préférence pour un accouplement hors du groupe de naissance, reconnaissance directe d'un apparenté, maturation sexuelle retardée chez l'un des sexes, rôle du système social… Cependant, le choix d'un partenaire optimal pour s'accoupler peut également être contraint par la disponibilité en partenaire de l'autre sexe dans le temps et dans l'espace, ou encore par des facteurs environementaux ou physiologiques. Peu de choses sont à l'heure actuelle connues concernant les différentes étapes qui précèdent l'accouplement, comme par exemple le lieu d'émergence des deux sexes, la probabilité de rencontrer un apparenté ou encore comment ces facteurs influencent le choix final de l'individu. Ceci est particulièrement le cas pour les insectes parasitoïdes où l'émergence des adultes est conditionée par le comportement de leur hôte.

Les parasitoïdes sont des organismes qui en parasitent d'autres et les tuent lors de leur dévelopement. Les Hyménoptères parasitoides sont haplo-diploïdes (le mâle est haploïde et la femelle diploïde) et, pour certains d'entre eux comme les parasitoïdes de pucerons (Aphidiinae), lorsqu'une femelle s'accouple avec un mâle apparenté, leur mode particulier de determination du sexe fait qu'elle donne naissance à une certaine proportion de mâles diploides, souvent stériles, en lieu et place de femelles. De plus, comme chez ces espèces les hôtes vivent en groupes (colonies de pucerons), quand une femelle pond ses oeufs au sein des pucerons de la colonie, les parasitoïdes qui en emergent quelques jours plus tard le font de façon synchrone. Ceci a pour consequence une compétition entre frères pour l'accès à leurs soeurs pour s'accoupler, compétition connue sous le nom de "Local Mate Competition". Mais comme ces parasitoïdes sont sensibles à la consanguinité, ils ont probablement développé des méchanismes pour l'éviter. Au sein de la sous-famille des Aphidiinae, très peu d'études se sont

intéressées aux conséquences de la consanguinité et à leur statut d'espèce quasi-grégaire.

Dans ce contexte, notre hypothèse de travail est que premièrement le comportement des pucerons doit influencer la structure d'accouplement de ses parasitoïdes. Deuxièmement, les parasitoïdes ont du developer des méchanismes leur permettant d'éviter un accouplement consanguin et de limiter la compétition entre frères sur le patch d'émergence.

Au cours de cette thèse, nous avons montré que le puceron *Myzus persicae* disperse de sa colonie quand il est attaqué par le parasitoïde *Aphidius matricariae.* Ce comportement des pucerons a pour conséquence une émergence des parasitoïdes plus solitaire (chacun sur sa feuille) que quasi-grégaire (plusieurs sur une même feuille de navet) (article 1). De plus, lorsque nous avons observé la cinétique d'émergence des adultes, nous avons montré que les probabilités de rencontrer un apparenté issu de la même ponte sont faibles : les mâles émergent souvent avant les femelles (protandrie) et la distribution des émergences issues d'une même ponte est étalée dans le temps (3 jours consécutifs) (article 2). De plus, les frères et les sœurs n'émergent que rarement ensemble (article 2). Puis, notre second objectif était d'évaluer quels étaient les traits liés à l'accouplement qui pouraient éviter un accouplement entre apparentés. Notre hypothèse d'une fenêtre d'accouplement différente entre les mâles et les femelles est validée (article 4), les femelles ayant besoin d'un temps de maturation sexuelle plus grand que les mâles et étant sexuellement réceptive moins longtemps (article 4). Cependant, contrairement à nos prédictions basées sur la littérature, les femelles ne s'accouplent qu'une seule fois, augmentant les risques liés à un mauvais choix d'un partenaire génétiquement trop proche (article 3). De plus aucun évitement d'un accouplement entre frère et sœur n'existe d'un point de vue comportemental (article 5). Ces deux derniers résultats sont probablement de reflet de la sélection de différents traits agissant avant la rencontre des partenaires qui favorisent un accouplement entre non apparentés.

En conclusion de cette étude, le parasitoïde de pucerons *A. matricariae* possède des stratégies et des traits d'historie de vie qui diminuent les probabilités d'accouplement entre un mâle et une femelle apparentés. Cependant, comme toutes nos observations ont été réalisées en laboratoire, une étude de terrain permettrait de valider nos résultats sur des populations plus naturelles et de les affiner.

Table of contents

General context of the study

Theoretical models of **sexual selection** predict that both males and females should benefit by selecting their mating partners (Darwin, 1871). Evolutionary biologists have come to regard the events surrounding mating as a set of intra- and inter-sexual battles, which reflect the reproductive interests of males and females, these interests being sometimes common, often differing (Choe & Crespi, 1997; Alonzo & Warner, 2000; Brown *et al.,* 1997). A fundamental question raised by the evolution of mating systems addresses the role of each sex in **mate choice**. This implies an understanding of how and when cues used to select mating partners are used. For a long time, female mate choice has been the paradigm and most studies on sexual selection still focus on the role of females because of their higher investment in gamete production and care of offspring (Darwin, 1871; Jennions & Petrie, 1997). However, recent evidence suggests that the importance of male mate choice has been underestimated (Bonduriansly, 2001). More recently, a growing number of theoretical models predict that members of both sexes should be selective when they incur similar reproductive costs, resulting in mutual mate choice (Servedio & Lande, 2006; Lihoreau *et al.,* 2008). Optimization theories are also applied to mate choice (Parker & Smith, 1990), as the optimal strategy for each sex depends on the behaviour of the other.

There are different kinds of benefits that animals may derive by choosing certain mates rather than others and these benefits may be realised more or less immediately (high fecundity or fertility, higher parental abilities, resource choice…). In many species, individuals base their mate choice on genetic relatedness (Simmons, 2000; Archie *et al*., 2007) to optimize genetic compatibility and to avoid costly inbreeding and/or outbreeding depression (Bateson, 1983; Tregenza & Wedell, 2000). The advantage for each sex to accept or to **avoid inbreeding** would thus depend, on one hand, on the strength of inbreeding depression and, on the other hand, on characteristics of the mating system, such as reproductive investment of both sexes (Bateson, 1983), but also on making the optimum choice.

Independently of the life history traits of a speices, habitat characterisctics are also of importance in the evolution of mating strategies. A suitable habitat may need to contain a mixture of patches that provides opportunities for all of the activities required for successful reproduction (food resources, mate opportunites…) (Shaw, 2006). The environmental characteristics (biotic and abiotic components) influence the population dynamics of the different species that are present in the habitat. The environmental characteristics will thus affect mating opportunities, the mate encounter rate, and the genetic diversity between individuals of the same species (van Emlen & Oring, 1977).

As a parasitic organism depends on another to develop, the evolution of its mating strategies is more complex and partially depends on its host's

habitat selection and behaviours (Barrett *et al.,* 2008). Hosts represent an inherently patchy and dynamic resource that varies spatially and temporally in the time available for infection. Furthermore, host species differ in life-history traits, such as longevity, mating system, and phenology, which also influences parasite population dynamics and mating strategies. Parasite mating strategies have thus evolved in function of the ecological characteristics of their hosts. Insect parasitoids are a useful model to study how the habitat used by a host could influence parasite life history strategies. Indeed, parasitoids are at the third level of the tri-trophic system composed of the host plant, the host, and itsef (Godfray, 1994). Its life history traits, particularly those linked to mating strategies will thus depend of the distribution of its hosts in the environment.

Insect parasitoids are organisms that develop in or on a host that will die as as a result of the parasitoids' development (Eggleton & Gaston, 1990). Despite this common life history strategy, parasitoids belong to various insect taxa (Godfray, 1994). Because parasitoids are dependant on a host to achieve their development, numerous parasitoid life history traits are strongly linked to those of their host (Godfray, 1994; Hochberg & Ives, 2000; Wajnberg *et al.,* 2008). For instance, the mating system of parasitoids is partially influenced by the spatial and temporal distribution of adults at emergence, which depends on the spatial distribution of the hosts and how the female exploits the host population (Godfray, 1994). Females of solitary species that lay eggs in isolated hosts produce adults that need to disperse from their natal patch to find a mate. In such species, competition for mates occurs throughout the whole population. Conversely, in solitary parasitoids that develop quasi-gregariously (i.e. when foundress females lay eggs in several hosts that are clumped) and gregarious parasitoids (i.e. when foundresses lay several eggs in a host), the offspring pupate and emerge at approximately the same time. Emerged adults thus have the opportunity to mate totally or partially in their natal patch before dispersal. In that case, there is a high probability of encountering a related mate, and consequently a risk of inbreeding.

Inbreeding risk depends also on the capacity of partners to recognize a related individual and to avoid mating. Considering these different outcomes, and assuming inbreeding reduces the fitness of the next generation, one can predict that selection has probably forced evolution towards a minimization of the encounter or mating of related individuals.

Within Hymenoptera, the various mechanisms of sex determination lead to various ways of being more or less sensitive to inbreeding and give the opportunity for selection on many adaptive traits favouring or not inbreeding avoidance. For instance, parasitoids of the superfamily Chalcidoidae, which include Trichogrammatidae species and *Nasonia vitripennis*, do not share the Complementary Sex Determination mode of

reproduction as in many other Hymenoptera species and are not sensitive to inbreeding (Godfray, 1994; Hochberg & Ives, 2000; Heimpel & De Boer, 2008). On the other hand, members of the Braconidae family possess species that use the Complementary Sex Determination model, thus making them sensitive to inbreeding. Therefore, different strategies should have evolved in Braconidae species to avoid sib mating and the deleterious effects of inbreeding depression. In some species of Braconidae, individuals are singly distributed throughout the environment. This distribution tends to naturally decrease the probability of sib mating within a population (Godfray, 1994). Other Braconidae species, such as aphid parasitoids, are potentially more clumped in the environment due to the ecological characteristics of their host, and are assumed to be under a higher inbreeding pressure when they emerge. For these last species, evidence of deleterious effects due to inbreeding has been observed in some aphid parasitoids (*Aphidius rhopalosiphi*: Salin *et al*., 2004), suggesting that some physiological or behavioural traits had been selected to favour outbreeding.

In that context, when host are clearly aggregated and when parasitoids suffer from inbreeding depression, how do individuals avoid sib mating? Our hypothesis is that two sets of mechanisms are involved. The first refers to the period before mating and is linked to the sapio-temporal distribution of adults at emergence. Our predictions are 1) parasitation of an aphid patch by an adult female changes aphid distribution and thus the place of adult parasitoid emergence, 2) protandry and dispersal at emergence reduce mating on the patch, 3) temporal distribution of emergence increases that phenomenom. The second set of mechanisms is linked to mating strategy itself. We first expect that males will preferentially choose to mate with a non-related female. Our second expectation is that females are likely polyandrous and accept mating throughout their lives, which may reduce the number of constraint females in the population. We use the generalist aphid parasitoid *Aphidius matricariae* as our model species.

After a review of the literature about the consequences of inbreeding and the mechanisms selected in animals to avoid mating between siblings, we review the parasitoid mating systems. The unifying thread of this review was the relationship between sex determination mechanisms, mate competition, and inbreeding avoidance. We then focus on aphid parasitoids, which are the model of our study and which belong to the Braconidae family, which contains both sensitive and non-sensitive species to inbreeding.

The central part of the study was then divided in 2 chapters corresponding to the following 5 manuscripts.

Paper 1: Bourdais D. & Hance T. Aphid dispersion after a parasitoid attack favour a solitary emergence of the female parasitoid offspring. *Submitted to Environmental Entomology*

Paper 2: Bourdais D. & Hance T. Emergence rhythms favour outbreeding in the aphid parasitoid *Aphidius matricariae* (Hymenoptera: Braconidae). *Submitted to Comptes rendus de Biologie*

Paper 3: Bourdais D. & Hance T. Shift in mating strategy with oviposition in *A. matricariae* females. *Submitted to Bulletin of Entomological Research*

Paper 4: Bourdais D., Mailleux A.-C., Jerbi E. M., Hance T. Mature mates still sexy in the aphid parasitoid *Aphidius matricariae. Submitted to Journal of Insect Physiology.*

Paper 5: Bourdais D. & Hance T. 2009 Behavioural Processes. 81:92-94

We finally discuss the implications of inbreeding avoidance in our model as compared to other parasitoid species.

Chapter I. Review of the literature

1. Why organisms do not mate randomly: From sexual selection to inbreeding

Reproduction is the biological process by which new "offspring" are produced from their "parents". It is thus a fundamental feature of all known life and each individual organism exists as the result of reproduction. Reproductive systems can be asexual, sexual, or a mixture of sexual and asexual modes.

1.1. Sexual selection

In order to explain the evolution of sexual traits in dioecious animals, Darwin (1871) introduced the notion of sexual selection, and defined it as “the competition or struggle in one sex, primarily the male sex, for the possession of the other sex”.

Sexual selection requires sexual reproduction: the combination of genetic material from two parents in the progeny (Andersson, 1994). Although sex is the most common mode of reproduction among eukaryotes, the advantages of sexual reproduction remain a major unsolved issue in evolution (Barton & Charlesworth, 1998; Otto & Lenormand, 2002). Indeed, sexual reproduction requires an important mobilization of energy, from the development of gametes to courtship and parental care.

The evolution of **anisogamy** (gamete dimorphism) is considered a crucial transition in evolution (Maynard Smith & Szathmary, 1995) because it represents the evolution of two sexes: males and females. The female sex produces relatively few, large, and usually non-motile gametes (eggs or ovules), whereas the male sex produces many, smaller, and often motile gametes (sperm or pollen). It is often assumed that males invest considerably less in gametes than females, but quantifying the energetic cost of gamete production in both sexes has remained a difficult challenge. Indeed, for a broad diversity of species (invertebrates, reptiles, amphibians, fishes, birds, and mammals), investment in gonad biomass is nearly proportional to body mass in both sexes, but gamete biomass production rate is approximately two to four orders of magnitude higher in females (Hayward & Gillooly, 2011). Moreover, parental investment is often higher than the gamete production itself, increasing the complexity of the anisogamy model (e.g. parental care of both sexes).

Research on sexual selection has contributed to important insights in

evolutionary research, but it has also been subjected to controversy and debate since Darwin (1871). Much of the debate has been devoted to the relative importance and degree of sexual selection in and between the two sexes (Brown Blackwell, 1875 cited in Clutton-Brock, 2007). Sexual variation is traditionally understood as differences between males and females in one particular trait. However, during the past two decades, we have witnessed the discovery of widespread variation among individuals within each sex (Taborsky, 1994) called alternative strategies (Gross, 1996).

The act of mating can deviate from a random expectation in several ways. Indeed, sexual selection can occur whenever there is non-random mating and/or fertilisation, and results from **mate choice** or intra-sexual competition for mates. The distinct reproductive roles of males and females, which for many years were characterised in terms of competitive males and choosy females, have remained a central focus of sexual selection since Darwin's time. Indeed, individuals sharing a phenotypic trait may mate with each other more or less often than expected at random. The **degree of inbreeding and outbreeding** within a species is thus an important selective pressure that acts on mating systems.

1.2. Mate choice: having sex, yes, but with whom?

Mate choice may be defined as "any pattern of behaviour shown by members of one sex, that leads to their being more likely to mate with certain members of the opposite sex than with others," (Halliday, 1983) or as a differential sexual response to different types of reproductively mature conspecifics of the opposite sex (Bondurianski, 2001). This behavioural mechanism is central to the theory of intersexual selection as proposed first by Darwin (1871) and then reinforced by Fisher (1930).

A dominant perspective has been one of indiscriminate males competing for the attention of choosy females (Bateman, 1948). Much research has therefore focused on describing the roles and importance of male–male competition and female mate choice in driving evolutionary change (Andersson, 1994). However, it has become increasingly clear that male and female sex roles can be dynamic and variable. For instance, "reversed" sex roles, with male mate choice and female–female competition, were first identified in species in which females compete for access to males that contribute paternal care (Gwynne, 1991). Male mate choice has also been observed in species in which males do not make a significant contribution to offspring care (Bonduriansky, 2001).

Adaptive explanations for mate choice concentrate on the **benefits** that can be accrued by discriminating among potential reproductive partners. The benefits of mate choice can be classified as direct benefits or genetic benefits (see Jennions & Petrie, 1997; Andresson & Simmons, 2006). Direct benefits give a direct advantage in the case of reproduction (e.g. nuptial gift, large territory, parental care) (Moller & Jennions, 2001). Genetic benefits can result from selection for having sexy sons or through the good genes theory mechanisms. In that case, a trait is linked to a genetic advantage that will be transferred to the offspring. Female preference for such traits can provide genetic benefits to those of her offspring that inherit favourable alleles from their father (Andresson, 1994; Bonduriansky & Rowe, 2005; Neff & Pitcher, 2005).

Mate choice can also induce **costs** for the choosy sex. Costs can be due to the searching time (Deutsch & Reynolds, 1995; Jonhstone *et al.,* 1996), the risk of rejecting good mates (Parker, 1983; Real, 1990), the energy for searching for and assessing mates (Parker, 1983; Watson *et al.,* 1998), the risks of predation or parasitism (Crowley *et al.,* 1991; Rowe, 1994) or the risks of injury (Bonduriansky & Brooks, 1998).

However, the **optimum choice** for a mate will depend of the balance between the costs and benefits for a given individual at a given time. Indeed, life history theory is essentially concerned with identifying optimal solutions to various trade-offs, the most important of which are that between somatic effort (i.e., growth, maintenance, and learning) and reproductive effort. Within reproductive effort, many aspects should also follow the optimization rule (Parker & Smith, 1990). Indeed, the individual level of choosiness can also fluctuate with time reflecting individual strategic allocation of mating resources (Engqvist & Sauer, 2000).

1.3. Inbreeding in mating systems

1.3.1. What is inbreeding?

Inbreeding is the mating of related individuals that carry alleles identical by descent from a common ancestor or ancestors. Inbreeding depression is a decrease in the mean values of fitness-related traits in offspring due to the expression of recessive deleterious alleles, loss of overall heterozygosis, or emerging negative epistatic interactions between homozygous loci (Charlesworth & Charlesworth, 1987; van Oosterhout *et al.,* 2000; Roff, 2002).

1.3.2. Inbreeding consequences

Fitness and variations in some traits' expression are often affected by inbreeding: **egg-hatching rate** (van Oosterhout *et al.,* 2000; Haikola *et al*., 2001; Kruuk *et al*., 2002), **juvenile survival** (Armbruster *et al*., 2000; Haikola *et al.,* 2001; Nieminen *et al*., 2001; Fox & Scheibly, 2006), **development time** (Morjan *et al*., 1999), **increased developmental instability** (Roff, 2002. Reale & Roff, 2003), **lower female fecundity** (Henter, 1993; Roff, 1998; Saito *et al.,* 2000), **male fertility** (Saccheri *et al.,* 2005; Hughes, 1995) or **life span** (Henter, 1993; van Oosterhout *et al*., 2000).

1.3.3. Inbreeding avoidance mechanisms

Thus, fitness consequences of inbreeding exert selection on several traits that will affect mating patterns of males and females. Inbreeding avoidance is expected whenever the costs of inbreeding exceed the costs of inbreeding avoidance itself (Parker, 1979; Kokko & Ots, 2006). However, inbreeding depression is often severe enough to cause the evolution of inbreeding avoidance mechanisms in most of animals (Pusey & Wolf, 1996; Facon *et al.,* 2006).

Several types of mechanisms, including dispersal, extra-pair/extra-group copulation, recognition avoidance of kin/nestmate as mate, and polyandry (Cornell & Tregenza, 2007; Firman & Simmons, 2008) have been documented in various animal species (reviewed by Pusey & Wolf, 1996).

Dispersal is considered to be an inbreeding avoidance mechanism in many taxa (Waldman & Mckinnon, 1993; Pusey & Wolf, 1996; Bonte, 2009). Dispersal, in particular natal dispersal, is a frequent feature of animal life cycles (Perrin & Mazalov, 1999). Since long-distance movements through unknown environments are bound to incurr both mortality risks and energetic costs, the prevalence of dispersal must be maintained by some selective forces, such as inbreeding avoidance (Parker, 1983; Waser *et al.,* 1986). It has long been noticed that dispersal tends to be female biased in birds, while it is usually male biased in mammals (Greenwood & Harvey, 1978). Dispersal for inbreeding avoidance has also been reported in fishes (Hutchings *et al*., 2002), amphibians (Austin *et al.,* 2003), reptiles (Tucker *et al.,* 1998) and insects. However, the question of whether dispersal strategies have evolved as inbreeding avoidance or whether they result from intra-sexual competition or competition for resource is still debated (Pusey & Wolf, 1996).

Extra-pair copulations may be especially important in organisms for which dispersal opportunities are limited and therefore for which kin of

the opposite sex reside in the same group. This may apply to many birds and mammals in which shortage of breeding opportunities is widely cited as a cause of group living and cooperative breeding (Stacey & Koenig, 1990). In many birds, social monogamy restricts mate choice, but females have nonetheless been observed to pursue extra-pair copulations (Griffith *et al.,* 2002). Because habitat limits dispersal in Ethiopian wolves (*Canis simensis),* female wolves exploit the benefits of extra-pack copulations in order to avoid inbreeding without losing the security of group-living in a stable high-quality year-round territory (Sillero Zubirir *et al.,* 1996).

Some species are able to use **direct kin recognition** to avoid mating with closely related individuals. Experimental studies in a variety of species demonstrate that close relatives are often unattractive as mates (Blouin & Blouin, 1988; Pusey & Wolf, 1996; Facon *et al*., 2006). The ability to discriminate between kin and non-kin is better developed in species in which the mating system favours dispersal of both sexes. For instance, bumblebees can recognize and avoid mating with a female of the same nestmate (Foster, 1992). On the other hand, close relatives are often avoided as mates especially among animals that have a well-defined social structure (Pusey & Wolf, 1996).

In some other species, siblings failed to mate because **sexual maturation** was delayed. In a mammal example, female lions undergo œstrus at a younger age when the father has been replaced by a new male (Hanby & Bygott, 1987).

Inbreeding avoidance can also appear at a **post-copulation** level. For example, it has been shown that females of the field cricket, *Gryllus bimaculatus* De Geer, are able to selectively fertilize their eggs with sperm from unrelated males when they are allowed to mate with both related and unrelated males (Tregenza & Wedell, 2002; Simmons *et al*., 2006).

Some other strategies of inbreeding avoidance, mainly found in plant species, are associated with a **sex determination mechanism**. Hymenoptera have a special mode of sex determination that can have important consequences in terms of inbreeding. In the Hymenoptera, fertilized (diploid) eggs develop into females, whereas unfertilized (haploid) eggs develop into males. Deleterious alleles are usually purged in haploid males (Antolin, 1999; Zhou *et al.,* 2007), thus reducing the detrimental consequences of inbreeding relative to diploid species. However, many Hymenoptera species suffer considerable inbreeding depression (Antolin, 1999; Henter, 2003), by the occurrence of homozygote diploid males in species with the ancestral single-locus Complementary Sex Determination (sl-CSD; Cook, 1993; van Wilgenburg *et al.,* 2006; Heimpel & De Boer, 2008). Consequently, species with sl-CSD are expected to have evolved mechanisms of inbreeding avoidance to cope with the mutation load imposed by the production of diploid males (Cook & Crozier, 1995; Hein *et al.,* 2009).

2. Parasitoids mating systems

2.1. What is a parasitoid?

Reuter introduced the term parasitoid for the first time in 1913 to describe the strategy in which, during its development, the parasite first lives in or on the body of a single host individual, then kills that host, and as an adult parasitoid is free-living. The usual definition is now the one proposed by Eggleton & Gaston (1990): "*a parasitoid is an organism that develops on or in another organism called a host, from which it extracts its food and kills directly or indirectly at the end of its development*".

Parasitoids are among the most abundant of all animals, comprising some 10% of all metazoan species. They occur in seven orders: Coleoptera, Diptera, Hymenoptera, Lepidoptera, Neuroptera, Strepsiptera and Trichoptera.

In the world, 87000 species of parasitoids have been described. Within the Hymenoptera, they are about 45 families and 67 000 species (Eggleton & Belshaw, 1992; Godfray, 1994). Most of them are Hymenoptera. Modern treatments of Hymenoptera phylogeny recognize that the traditional suborder Symphyta (sawflies and woodwasps) is paraphyletic, whereas the suborder Apocrita is monophyletic (Hanson & Gault, 1995; Whitfield, 1998). Thereafter, however, free-living, ectoparasitic and endoparasitic species have arisen independently many times within and/or between each lineage (figure 1).

Insect parasitoids have been well studied through applied research because of their importance as biological control agents (Heimpel & Lundgren, 2000; Boivin *et al.,* 2012). Many species have been released and have successfully controlled economically important pests. These studies furnish a good database on the biology of many parasitoid species. More recently, behavioural ecologists have used parasitoids as model systems to study a variety of evolutionary questions (Godfray, 1994; Wanjberg *et al.,* 2008).

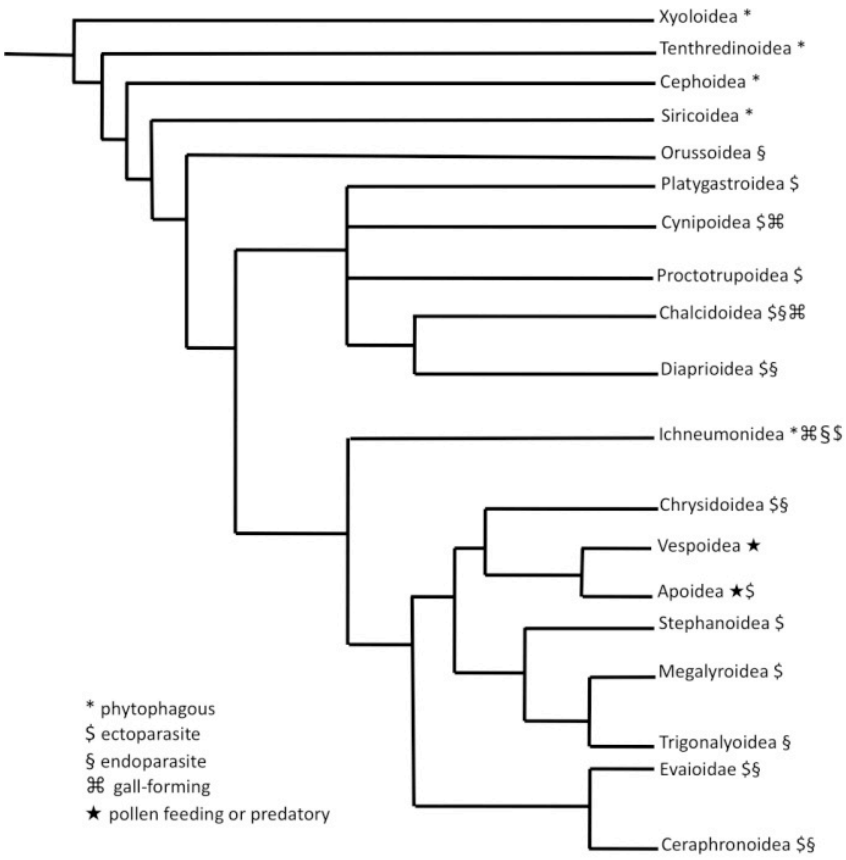

Figure 1. Consensus phylogeny and larval feeding habits for the Hymenoptera super-families. Modified from Hochberg & Ives (2000).

Parasitoids species can be classified through various ways:

- Parasitoids can be *egg*, *larval* or *pupal* parasitoids depending on the host stage they attack.
- Parasitoids can develop within the body of their host, feeding from the inside, and are known as *endoparasitoids*. *Ectoparasitoids* live externally, with their mouthparts buried in the body of the host.
- A *koinobiont* species keeps the host alive. *Idiobionts* prevent any host growth following parasitism and kill or paralyze at a long non-growing host stage.
- Solitary *vs.* gregarious. Another distinction is made between solitary parasitoids and gregarious parasitoids. However, because various definitions was reported in the literature, we decided to use the following in this document (figure 2):

- A *solitary* species is a species where one single adult emerges from one single host that is not distributed in patches in the environment.

- A *gregarious* species is a species where several adults emerge from one single host.

- A *quasi-gregarious* species is a species where one single individual emerges from one single host that is distributed in patches in the environment.

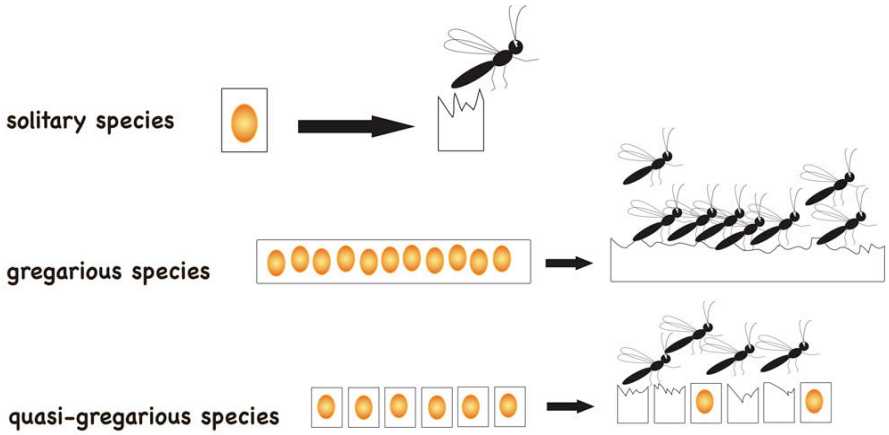

Figure 2. Schematic view of a solitary, gregarious and quasi-gregarious parasitoid species in relation to the moment of adult emergence. The host is represented by a square and the parasitoid's egg by the yellow circle. ©D.Bourdais

2.2. The patch problem

When investigating questions related to parasitoids, the notion of "patch" can lead to confusion and misleading interpretations.

Parasitoid hosts are sometimes distributed randomly in the environment but are more often distributed in discrete patches. The behaviour of parasitoids searching for patchily distributed hosts has attracted considerable attention from parasitoid biologists, but also from theoretical behavioural ecologists (see inset). In this section, we do not review studies and theories about patch use in parasitoidsbut rather ask the question: what is a patch?

A general difficulty in the empirical study of parasitoid aggregation is the **delimitation of a host "patch"**, and therefore, the determination of local host density, in a biologically meaningful way, i.e. in a way that

mirrors the parasitoid perception (Rosenheim *et al*., 1989). Waage (1979) has unambiguously defined a host patch for parasitoids as a host that generates, or is associated with, a localized arresting stimulus, which may be a physical structure or a contact semiochemical. In these cases, the functional area of the arresting stimulus defines a patch. However, such a definition cannot be applied universally, due both to our ignorance of potential arresting stimuli and to the absence of such stimuli from many systems (van Alphen & Vet, 1986).

Patch exploitation strategies: definitions and concepts.

The classical patch-use models predict when an animal, foraging alone, should leave one patch and begin searching for another. When the animal enters the patch, its rate of gain of fitness is initially high but then drops as the patch is depleted. **Charnov (1976) marginal value theorem** shows that the optimum time to leave a patch is when the instantaneous rate of fitness gain drops to the maximum average rate that can be achieved in that environment. Then, factors that reduce the maximum rate of fitness gain in that environment lead to greater patches residence time.

However, this model has been criticized and the patch-leaving model that has the most influence in studies of parasitoids searching is due to **Waage (1979)**. He assumes that a parasitoid responds to chemicals secreted into the patch by hosts, and leaves the patch when its responsiveness drops to zero. On entering the patch, the responsiveness is set at a certain value that decays linearly over time unless a host in encountered (Godfray, 1994; Wajnberg, 2006). What renders the system more difficult to analyse is that the female parasitoids allocate the sex of their progeny based on competition among mates (Hamilton, 1967), host quality (size, species, and sex) (Charnov, 1979), position in oviposition sequence (Suzuki *et al.,* 1984; Wajnberg, 1993), or population's sex ratio (Rotary & Gerling, 1973; Werren & Charnov, 1978). These aspects increase the level of complexity of the model.

Patch definition: a landscape point of view

The insect interpretation of what constitutes a patch depends on the scale.

As Addicott *et al*. (1987) pointed out, the correct scale for investigation depends on the movement patterns of the organisms involved. If, for example, a parasitic Hymenoptera searching for Lepidoptera hosts on trees moves only between leaves on a single tree, then the appropriate scale for investigation of density dependence would be single trees. The parasite would be unlikely to respond to large differences in density of hosts between

widely separated trees and a patch of hosts could be one leaf of the tree. However, for the study of a very mobile parasite that flies easily from tree to tree, a larger scale would be appropriate (Stiling *et al.,* 1991).

Organisms respond to information from the environment through their sensory system. The specific perceptual environment is referred to as the "*Umwelt*". Thus, the analysis of the information used by animals is considered a central field in ecology (Giraldeau, 1997; Dall *et al.,* 2005; van Dyck, 2012). The spatial scale of interaction between landscape structure (resource distribution) and the perception of the organism is referred to as the functional gain of a landscape (Baguette & van Dycke, 2007).

Moreover, the spatial scale and the notion of suitable habitat for foraging in parasitoids is dependant on its ability to disperse. For instance, *Anagrus delicates* (Hymenoptera, Mymaridae), an egg parasitoid, disperse by flying for more than 1km to search for hosts (Antolin & Strong, 1987). Another, *Elenchus koebeli* (Sterpsiptera) cannot fly and disperses from less than few meters. The functional scale is also dependent on habitat characteristics such as the structure or diversity of the vegetation (Bottrell *et al*., 1998; Casas & Djemai, 2002; Gols *et al.,* 2005; Obermaier *et al*., 2008).

A recent study of Zhao *et al.* (2012) estimates the minimum amount of suitable habitats (MASH) that can sustain a viable population in an aphid-parasitoid complex in wheat fields. The MASH indicates the lower bound of the interaction between the focal species and other species (or resources) on lower (or higher) trophic level. They concluded that when the patch size becomes larger, the population density becomes more stable. The parasitoid MASH was 479m^2, which is higher than for aphids (246m^2). These results show that the host-parasitoid interactions work only at 500m^2. However, it does not provide an answer to the size of a patch in aphid parasitoid complex.

Patch definition for foraging females

For parasitoids, a patch is defined as a spatial subunit of the foraging area in which aggregations of hosts occur (Wajnberg, 2006). Examples of patches are aphid colonies for aphid parasitoids or host egg masses for egg parasitoids (Wajnberg, 2006). Patches of hosts are usually of different quality (e.g., in terms of the number of hosts to attack) and, thus, of different profitability for the foraging females. In insect parasitoids, optimal foraging models have been applied mostly to host exploitation, with a patch as a single host (case of gregarious parasitoids) or a given number of clumped hosts (quasi-gregarious species) (Boivin *et al.,* 2004; Rosenheim *et al.,* 1994; Vos *et al.,* 1998; Weisser *et al.,* 1994; Goubault *et al.,* 2005, etc… but see Wajnberg, 2006 for a review). It thus seems that the definition of a patch for a females is quite clear (Godfray, 1994) in lab studies. From a

behavioural point of view, the patch leaving behaviour expressed by the female is often associated with a dispersal flight from the patch (Wajnberg, 2006).

One field study reports that the distances traveled between stems in *Aphidus ervi* females parasitizing *A. pisum* aphids ranges from 5 cm to 20 cm (Olson *et al.,* 2000) and authors lose the visual contact with female when they move more than 1 meter by flying. This shows that a female is foraging in a 20 cm radius by walking and leaves their "patch" through dispersal if no hosts were encountered.

Patch definition for males (example of Trichogramma *males)*

Martel *et al*. (2008) suggested that the optimal models of female patch exploitation strategies could fit with male mating strategies. Males should also obtain higher lifetime fitness by leaving a patch at an optimal moment rather than staying to mate all available females. The fitness gain of a male parasitoid is obtained through the number of daughters it produces. The fitness gain is thus influenced by the number of females mated but also by the quality of these females (i.e. their fecundity, longevity, mating status, etc.) and by the quantity of sperm transferred at each mating. As a result, male parasitoids, and not only females, should use strategies that optimise their mate acquisition throughout their life to maximise their lifetime progeny production.

Under the mating structure with Local Mate Competition, mating occurs both on- and off-patch (Hardy, 1994). This occurs in many species (Kazmer & Luck, 1991; West & Herre, 1998; Gu & Dorn, 2003). For these males, time can then be a limiting factor and behaviours should tend to optimise time allocation by maximising the number of females inseminated. How much time to invest in a patch before leaving is therefore a question as important for males as for parasitoid females. Martel *et al.* (2008) concluded that the information used by Trichogrammatidae males to decide when to leave the patch is thus the number of virgin females and the number of host eggs contacted, either emerged or not. However, further studies are necessary to look at the functional aspects of male patch allocation. Nevertheless, the definition of a patch seems clear in these species and is related to the definition of a patch for a female (a clumped group of eggs). Unfortunately, this is the only study we found on the notion of patch applicable to male mating strategies.

To locate potential mates, male parasitoids are known to use volatile sex pheromones (McNeil & Brodeur, 1995) and other information to locate sites where females aggregate to feed and forage for hosts (Nadel & Luck, 1992; Godfray, 1994; Gu & Dorn, 2003; Bezemer *et al.*, 2010). However, we have no precise data about the distances they can fly and how they use chemical information in mate searching.

Our patch definition in this study on mating strategies of aphid parasitoids

To date, no clear response exists on defininig a patch, especially when males and females explore their environment for mating. In this study, we often use the word quasi-gregarious to qualify aphid parasitoids. This is the definition used by several authors (Godfray, 1994; Maukauer & Volkl, 2004; 2005; He & Wang, 2008). Aphids are clumped in colonies and the distance between colonies can vary within year, depending on the population levels of aphids. Thus, the scale of a patch can vary for the searching parasitoid. Nevertheless, a patch can be defined as a local area in which the parasitoid modifies its research behaviour, for instance by flying instead of walking (Wanjberg *et al.,* 2008; Vos *et al.,* 1998; Martinou *et al.,* 2009). When the patch quality is too low, it may be worthwhile to leave the patch and accept the cost of inter-patch travelling in order to try to find a better patch (Pierre *et al.,* 2003). However, the decision to emigrate to another patch could be costly because the flying parasitoid is more exposed to predation but may also be unable to find another suitable patch.

Here, we consider a **clumped aphid colony** as a patch, by comparison to what happens in quasi-gregarious parasitoids with immobile hosts (e.g. egg parasitoids). This scale was chosen for the following reasons:

- Hosts can move from their initial colony from few centimetres to several meters. In each case, if parasitized aphids move from the initial place, they will be less clumped than in a patch of Lepidoptera eggs. Thus, parasitoid adults that emerge from the aphids will be under lower pressure from Local Mate Competition than in egg parasitoids or gregarious ones. It does not mean that there is no Local Mate Competition, but newly emerged individuals need to disperse (even of few centimetres) toward an attractive area (for instance a virgin but sexually mature female) to mate.

- We do not know the precise distance of attraction of males towards females in field, probably because it depends of so many biotic and abiotic factors and the laboratory response to female sex pheromones does not preclude mate attraction for long distances in field conditions. In the same approach, we do not know how far such a small parasitoid male can disperse by flying or walking toward an attractive odour.

By comparison with egg parasitoids, emerged offspring of aphid parasitoids will be more or less clumped depending on how far the parasitized host moved from the original patch.

Field investigations are important to conduct to better understand the scale of a patch for a foraging male of an aphid parasitoid. But for comparitive purposes, we decided to use the same definition as for females

(one patch is one aphid colony) and other quasi-gregarious males (one patch for LMC is the same, the initial aphid colony).

2.3. Mating system of parasitoids

The diversity of sexual and asexual modes of reproduction in insects creates a corresponding variety in mating systems involved. We define in this work that a **mating system** specifies which environmental, abiotic and/or genetic factors rule the system; which males mate with which females, under which circumstances (e.g. in time, space…), or in other words "*how males and females obtain mates in a population*" (van Emlen & Oring, 1977; Davis, 1991; Brown, 1997; Thornill & Alcock, 2001).

There are various ways of classifying insect mating systems, one of which is the classification proposed by Thornill & Alcock (2001) andis based on a single criterion: *the number of copulatory partners per individual that is the one most likely to compete for members of the opposite sex* (Reynolds, 1996; Table 1).

A classification for mating systems based on number of mates per individual can be used at either the individual level or the population level. At the population level, one can characterize an entire species as polygynous if a percentage of at least 5 to 10 percent of the males acquire several mates (Thornill & Alcock, 2001).

Table 1. Various types mating systems in relation to the number of partner

	Number of copulation per mating season	
	1	>1
Male	Monogynous	Polygynous
Female	Monoandrous	Polyandrous

However, parasitoid mating systems are classified slightly differently than other insect species and are thus traditionally concerned with the issue of whether **mating is restricted and local or random and panmitic** (classification of Godfray & Cook, 1997) and the **genetic relationship between mates**.

To be concise, the mating system of a parasitoid species is influenced by:

1/ The **spatial and temporal distribution** of hosts (including the host characteristics) and newly emerged individuals in the environment. Being a specialist or generalist is also of importance

2/ The **dispersal** capacity of individuals

3/ Some aspects of the **reproductive biology** and **tactics of both sexes**: Timing of mating, mating frequency and use of sperm in case of multiple mating, mate choice…

4/ The sex determination rules and **resistance to inbreeding**

2.3.1. Spatial and temporal distribution of hosts and adult parasitoids.

Temporal and spatial distributions in the emergence of adult parasitoids are vital aspects of their mating systems because they determine mate availability. We can expect that **solitary**, **gregarious or quasi-gregarious** species are characterized by different life strategies: sex-ratio (defined as the % of males) of the brood, the level of inbreeding within a brood, or competition between males for access to females (see figure 2).

The Local Mate Competition Theory and its consequences on the sex ratio

The evolution of sex ratio is a major area of research because it influences population growth and because male reproduction has an actual cost. Moreover, in Hymenoptera, as they are haplodiploid species, the sex of the egg could be under the direct behavioural control of the mother. The sex allocation of foraging females depends on numerous internal and external factors (Godfray, 1994). Consequently, the degree of Local Mate Competition (LMC) may vary and we could expect that some mechanisms play an important role to limit competition in parasitoids.

Females of solitary species lay a single egg in evenly distributed hosts and competition for access to mates may occur throughout the whole population. In such species, Fisher's argument predicts a sex ratio of 50% of males (Fisher, 1930). Although sex ratio equality is observed in probably most of animal species, parasitoid wasps frequently have extremely female-biased sex ratios. Conversely, in solitary parasitoids that develop quasi-gregariously and gregarious parasitoids, brothers of the same brood compete for a limited number of females, through a process called **Local Mate Competition** (Hamilton, 1967; Franck, 1990; Antolin, 1993). This competitive disequilibrium between sons and daughters constitutes a bias from the Fisher model, which drives new predictions for the sex ratio. It assumes that a female has control over the sex ratio and can maximize her fitness by reducing the competition between her sons. This is an evolutionary stable strategy if males are not the dispersing sex and if mating only takes place at the natal patch (Hamilton, 1967). But we know that it is not the case in most of species and that is why this model has received many modifications (Godfray, 1993).

Since the first theory proposed by Hamilton (1967) where he described sex ratio under full local mating structures, many models have included modifications, such as the influence of partial local mating and the influence of host quality or number of foundress (review in Hardy, 1994).

According to these models, the **predicted sex-ratio (% of males) should decrease with decreasing number of foundress** from 50% (if the local population is so large that panmixis is possible) to next to 0% (in reality just the proportion of males that is necessary to copulate with all females of the patch). If multiple females oviposit in the same patch, their sons will compete for mating opportunities not only among themselves but also among non-siblings. This makes it adaptive for females to produce relatively more sons in the presence of another ovipositing mated female. Thus, as the number of ovipositing females (n) in a patch increases, the optimal sex ratio (r % males) should increase (Hamilton, 1967). The equation predicts a daughter-biased sex ratio for low numbers of foundresses, with increasing proportions of sons, as the number of foundresses in a patch increases, reaching an asymptote at a 1:1 sex ratio (50% of males).

However, predictions for sex ratio optimisations have to deal with some particular aspects of specific parasitoid behaviours. For instance, the classical assumption described before is not applicable to *Mellitobia spp* (Hymenoptera, Eulophidae), which are gregarious parasitoids where lethal male combat is known to occur. In that case, Abe *et al.* (2003) proposed a new model that takes into account the male combat and the parasitism order which determines the mating success of sons produced by each foundress. The observed sex ratio is highly female biased, independently of the number of foundresses.

Moreover, the assumption of fully local mating is frequently incorrect. In many species, males are potentially capable of dispersal from the natal patch, and virgin females may also disperse. **Partial local mating** is thus the rule in most of gregarious or quasi-gregarious species (Hardy, 1994; Godfray, 1994).

However, females do not always adjust the brood sex ratio according to the LMC theory. Two other major components exist and can influence brood sex ratio.

First, **competition for host resources** among developing larvae may alter sex ratio. For instance, when several female *N. vitripennis* lay eggs in the same host, larval sons and daughters engage in local resource competition (LRC), often causing greater mortality of daughters (Suzuki *et al.,* 1998; Godfray, 1994; Santolamazza-Carbone & Rivera, 2003). The effect of Local Resource Competition among larvae is one of the most important confounding factors in experimental studies of LMC (Godfray, 1994). In quasi-gregarious species, this aspect could be controlled in a

laboratory study, providing the same host quality to each host of the patch exploited by females.

Further, the **size of a female** parasitoid may affect her clutch size and the sex ratio of her offspring. Larger females tend to hold more eggs and produce larger clutch sizes than smaller females (Visser, 1994; Sagarra *et al.,* 2001; Santolamazza-Carbone *et al.,* 2007). Smaller *N. vitripennis* foundresses, who contribute a relatively low proportion of offspring to a patch, produce proportionally more sons because their sons are not likely to compete among themselves for mates and thus they are less affected by LMC (Werren, 1980).

Nuney & Luck (1988) provided a series of modifications to Hamilton's model, including the **probability of male dispersal (Partial LMC)**. This model predicts that as the probability of males finding patches after dispersal increases, the optimal sex ratio also increases towards equality. In that model, the effect is especially apparent when foundress number is small (<6; Hardy, 1994).

Independently of sex ratio models, one of the most important **consequences of LMC** is the **sib-mating opportunity** for males and females both in gregarious and quasi-gregarious species. If mating does not occur randomly among the offspring of foundresses, and in the presence of **inbreeding avoidance,** the degree of local mate competition among males is decreased and the optimal sex ratio is consequently less female biased (Stubblefield & Seger, 1990). However, if foundresses do not arrive at patches simultaneously, progeny emergence will be asynchronous in quasi-gregarious species, favouring sib matings. Thus, a new contribution of the model was developed by Nunney & Luck (1988) and predicted that asynchronous brood maturation leads to an increase in female bias.

In conclusion, mating structure is important to optimal sex allocation theory (but also host quality). All models that have explored mating structure intermediates between panmixis and fully local mating have found that non-local mating can have an important influence on the ratio strategies (Hardy, 1994). Generally, the greater the degree of local mate competition, the more the female biased the sex ratio. Less local mating leads to a higher proportion of males developing on poor quality resources. The prevalence of local mate competition is decreased by increasing foundresses' number, non-sibling matings and non-local matings (Hardy, 1994).

With the sex ratio of haplodiploid wasps affected by inbreeding, LMC, LRC, foundress relatedness, and/or the size of females, we have to incorporate these aspects in lab studies and be able to isolate the effect of each one of these factors. Moreover, some of the behaviour we observe may result both in inbreeding avoidance but also in LMC avoidance, for instance if the quasi- gregarious distribution of the parasitized host is disturbed during

parazitation process.

Mating system of solitary species

In solitary species, the adult parasitoid emerges alone from a single host that is not patchily distributed in the environment. According to assumptions of the sex ratio theories, no local mating can exist in these species and one should observe sex-ratio equality (Fisher 1930).

In such species, different strategies, such as pheromone production, have evolved to favour the encounter rate of males and females. However, male parasitoids tend to search for females rather than females for males because of two main reasons: (1) the operational sex ratio (the ratio of males and females, which are ready to mate at a given time) is often male biased and (2) Hymenoptera females do not have to mate in order to produce males and virgin reproduction may not be always a disadvantage (van Emlen & Oring, 1977). In solitary species, mating can occur at arbitrary sites or at feeding and/or oviposition sites and often depends on chemical attractants.

Mating system in gregarious species

When adult parasitoids are gregarious, many individuals, often siblings, emerge from a single host (see figure 2).

- Mating occurs exclusively on the emergence site

To our knowledge, no example of a gregarious parasitoid that mates exclusively in the emergence patch had been found. In all studies in the literature, at least a small proportion of males and/or female disperse before mating.

- Mating occurs mainly on the emergence site (partial local mating)

Nasonia vitripennis (Walker) (Hymenoptera, Pteromalidae) is a gregarious parasitoid attacking pupae of several fly species. The species has been intensively investigated in studies addressing genetics (Reed, 1993), ecological (King & D'Souza, 2004), behavioural (King & Skinner, 1991; Baeder & King, 2004), developmental (Rivers *et al.,* 1999) and evolutionary aspects (van den Asssem & Jachmann, 1982). The mating system of the species is characterized by protandry: males emerge first and females follow shortly after. Pheromones play an important role in sexual communication (Steiner *et al.,* 2006) and arrestment. Some key elements of the male courtship sequence are mediated by a female derived contact sex pheromone.

In this species, males and females mostly mate on the emergence patch but virgins may disperse (Hardy, 1994; Grillenberger *et al.,* 2008).

Cotesia glomerata (Hymenoptera, Braconidae) is also a good example of a gregarious parasitoid that mates mostly on the emergence patch (Gu & Dorn, 2003). *C. glomerata* is native to Europe where its principal host is the large white butterfly *Pieris brassicae* (L.), but it can also develop in *Pieris rapae* (L.). In 1883, it was introduced into the United States as a potential biological control agent of this host (Swan, 1964). The sex-ratio of most broods is female biased even if only males compose some broods. In mixed broods, males usually emerge before females and observed mating took place within a few minutes of female emergence (Tagawa & Kitano, 1981), with matedfemales emigrating shortly after. In this species, about 70% of matings occur on the emergence patch (Gu & Dorn, 2003) and less than 5% of females disperse when still virgin.

- Mating almost never occurs on the emergence site (partial local mating)

Bracon hebetor (Hymenoptera, Braconidae) is a cosmopolitan gregarious ectoparasitoid of pyralid moths (Lepidoptera, Pyralidae). Once having encountered a suitable host, the female lays about 10 eggs per host and larvae pupate in a group next to the consumed remnants of the host. Upon emergence, adults walk over the adjacent cocoons for a few minutes before dispersing (Antolin & Strand, 1992). The receptivity to mating for both sexes increases with age and some individuals avoid mating before dispersal. In this species, Ode *et al.* (1995) found brood mate recognition and sib-mating avoidance, behaviours that enhance mating out of the natal patch.

Mating system in quasi-gregarious species

Some parasitoids species are "physiologically" solitary during their development because only one adult can emerge from one host, but behave as gregarious species as adults because of the patchy distribution of their hosts.

- Mating occurs exclusively on the emergence site.

One good example of these species is the egg parasitoid *Trichogramma dendrolimi* (Hymenoptera, Trichogrammatidae) that is a quasi-gregarious egg parasitoid of Lepidoptera eggs. In this species, full local mating is observed (Suzuki & Hiehata, 1985); all females are inseminated before their emergence from the host. A lack of courtship and

short copulatory period is observed and adult wasps remain inside the host for a relatively long period before emerging.

- Mating occurs mainly on the emergence site (partial local mating).

Most *Trichogramma* species do not mate exclusively on the natal patch (Martel & Boivin, 2005) but the proportion of females that disperse still virgin is generally low. In a recent study, Martel *et al.* (2007) found that only 0.67% of females of *T. turkestanica* and about 6% of males disperse still virgin. In these species, the propensity to emigrate is lower for males than for females and males tend to stay longer on the emergence patch when females are present (Forsse *et al.,* 1992; Martel *et al.,* 2007). It is often males that disperse still virgin (Martel & Boivin, 2005) and such dispersal could enable males to mate with emerging females of other patches (Nunney & Luck, 1988).

One other example of quasi-gregarious species is the pupal parasitoid *Pachycrepoideus vindemiae* (Hymenoptera, Pteromalidae). Since males are long-lived and capable of flight, they usually emerge one day before and wait next to a female pupae and mount her just after its emergence (Nadel & Luck, 1985). Males also emigrate from broods and search for other broods to wait for females.

In *Spalangia cameroni* (Hymenoptera, Pteromalidae), a pupal parasitoid of flies, sib-mating is the result of the coincidence of emergence of both sexes because males usually disperse from the natal patch before female emergence (Myint & Walter, 1990) and females also disperse rapidly. Moreover, it seems that hosts containing females are attractive for males (Myint & Walter, 1990) that are searching for emergence sites.

Finally, aphid parasitoids (Hymenoptera, Braconidae, Aphidiinae) are also considered to be a good example of quasi-gregarious species with partial local mating (Schwörer & Völkl, 2001; He & Wang, 2008). As aphids are patchily distributed in colonies, a parasitoid female exploits host patches, laying eggs nearly at the same time in several hosts, and the progeny emerges simultaneously from the aphids of the colony. In that case, sib-mating should be expected and partial local mating should occur. To date, no reference about post emergence behaviour in relation to partial local mating behaviour in aphid parasitoids has been published.

2.3.2. Parasitoid dispersal

There are many definitions of dispersal in the literature (Clobert *et al.,* 2001). Here, we use the definition of Turchin & Omland (1999): "*dispersal is a form of population redistribution that involves the spatial spreading of its members*". Such movements by insects over varying distances are important for both theoretical (understanding movements between populations) and applied (conservation or monitoring of invasive species for instance) aspects. For parasitoids, the data published concern mostly short-range (<10km; Byrne, 1999) dispersal by flight because of its particular importance in landscapes where favourable patches are often found in proximity to one another.

Most studies on parasitoid flight capability were done on post-emergence dispersal or orientation of female to odours in laboratory experiments (Potting *et al.,* 1999; Daza-Bustamante *et al.,* 2002; Mc Clure & Mc Neil, 2009). Female *C. plutellae* can discriminate in-flight between odours emitted from un-infested leaves and leaves infested with, or damaged by, its host *P. xylostella* (Potting *et al.,* 1999). Wind tunnel flight responses (oriented flights of 80cm maximum - the maximum length of the wind tunnel) of *A. ervi* females from either alfalfa or wheat were significantly stronger towards the host plant complex on which they had been reared during the last generation before the experiments (Daza-Bustamante *et al.,* 2002). In *Trichogramma* species, males tend to stay longer before dispersing from the natal patch when females are present (Forsse *et al.,* 1992). A study of Martel & Boivin (2004) on three species of *Trichogramma* showed that 90% of mated females had dispersed from their natal patch between 3 and 4 hours after emergence. There was no result for males as few of them disperse (Martel & Boivin, 2004). However, all these studies were made under laboratory conditions that impose the study of only small potential dispersal distances.

Very little is known about adult parasitoid dispersal distances in field conditions because measuring the movement of small insects presents formidable technical problems (Mills *et al.,* 2006). Since Berry *et al*. (1972) suggested using rubidium to mark insects, trace elements have increasingly been used to mark small arthropods to study their movements in agro-ecosystems (Graham *et al.,* 1978; Muratori *et al.,* 2000; Prasifka *et al*., 2001; Pickett *et al*., 2004). Rubidium has been used in parasitoid mark-recapture studies (Muratori *et al.,* 2000; Scarratt *et al.,* 2008). For instance, *Anagrus sp.*, a grape leafhopper parasitoid, was found to move 100m downwind colonising adjacent grape winyards (Corbette & Rosenheim, 1996). Weisser & Wolkl (1997) examined between patch movements by the aphid parasitoid *Lysiphlebus cardui* Marshall and found that it moved no more than 20m

when searching for hosts. Trichogrammatidae parasitoids were found to have moved only 15m in 3 days when released in apple orchards (McDougall & Mill, 1997). Studies on aphid parasitoids of the genius *Aphidius* have shown that females disperse around 10m under natural conditions (*A. colemani*: Langhof *et al.,* 2005; *A. rhopalosiphi:* Muratori *et al.,* 2000, *A. matricariae* and *Ephedrus cerasicola*: Dumont *et al.,* 2009).

Wind might have a great effect on dispersal of such small insects and dispersal could be more passive than self-directed. A study by Bellamy & Byrne (2001) experimented the dispersal of *Eretmocerus eremicus* (Hymenoptera, Aphelinidae) under both lab and field conditions. They found that flight duration in the lab was influenced by gender and mating status. Females flew longer than males and unmated flew longer than mated ones. It is suspected that this behaviour is driven by a search for resources (Bellamy & Byrne, 2001). In the same study, they reported that males dispersed more than females in natural fields. Males do not have a directional distribution of dispersal within a 5m annulus around the release point but beyond 5m, the dispersal is wind directed. The results tend to show that both sexes abandon searching for resources near the release point and engaged in wind directed dispersal to locate more distant hosts or partners.

2.3.3. Behavioural and physiological aspects influencing the mating system

To mate successfully, insects have to solve problems such as locating a partner or performing the adequate behaviour to obtain an effective insemination. Males must take measures to enhance a sperm monopoly in fertilization. Mating behaviour of a particular species could thus be divided in **pair formation**, **courtship**, **copulation** and **post-copulatory events** (Alexander *et al*., 1997) and the differences of these behaviours should characterize a particular mating system.

Pair formation

When potential partners do not emerge simultaneously in space and time, chemical, acoustic, tactile and visual stimuli are involved in mate finding (see Thornill & Alcook, 2001; but also Eller *et al.,* 1984; Field & Keller, 1993; McNeil & Brodeur, 1995; Pompanon *et al.,* 1997; Fauvergue *et al.,* 1999).

- Chemicals are often volatiles and provide long-distance information about the signaller's location. Many females produce sex pheromones to attract males but the reverse is also possible (Eisner & Meinwald, 1995; Eisner *et*

al., 1996; Kainoh, 1999; Thornill & Alock, 2001; Steiner *et al.,* 2006). Chemicals can also be located in the environment. For instance, some females lay down trails of sex pheromones (Kainoh & Oishi, 1993; Fauvergue *et al.,* 1995; Kazmer *et al.,* 1996; Pompanon *et al.,* 1997; Fauvergue *et al.* 1998a) where males respond to an encounter with the pheromone by intensively searching in or near the marked area. Plant volatiles are also important cues in long-range attraction and mate location (McAuslane *et al.,* 1990; Ruther & Steidle, 2000; Jyothi *et al.,* 2002; Stelinski & Liburd, 2005).

However, it is important to precise here that chemicals also present some importance in the case of gregarious and quasi-gregarious species, especially in case of partial local mating strategies. Here we present data about the use of sexual pheromones in solitary species but also in gregarious or quasi-gregarious species.

Mating occurs at arbitrary sites: attraction by pheromones.

When parasitoids emerge solitarily, the use of female sexual pheromones is a clear advantage to optimize mate encounters. Pheromones are also common in quasi-gregarious or gregarious species. Many receptive female parasitoids release volatile sex pheromones that attract males from long distances (Godfray, 1994). In the case of solitary species, the use of volatile pheromones allows males to orientate to receptive females in the environment (Decker *et al*., 1993; McNeil & Brodeur, 1995; Nazzi *et al.,* 1996; Sullivan, 2002). If females are monogamous, the production of pheromones stops after mating (Fauvergue *et al.,* 1999) but in the case of polyandrous females, the production continues (DeLury *et al.,* 1999). At a shorter range, males often use chemical cues for mate recognition (McNeil & Brodeur, 1995; Kainoh, 1999; de Freitas *et al.,* 2004; Ardeh *et al.,* 2004; Steiner *et al*., 2006). In some cases, the origin and constitution of the chemical is known or at least suspected (Weseloh, 1976; McNeil & Brodeur, 1995; Syvertsen *et al.,* 1995; Finidori-Logli *et al.,* 1996; Nazzi *et al.,* 1996; DeLury *et al.,* 1999; Marchand & Mc Neil, 2000).

Very often males are attracted in a short range to insects of their own sex (Field & Keller, 1993; Steiner *et al.,* 2006; pers. obs on *A. matricariae, A. rhopalosiphi* and *A. ervi*).

Males search for feeding or oviposition sites: use of kairomones

Several studies have investigated the possibility that males seek mates at oviposition sites (van Dijken *et al.,* 1989; Myint & Walter, 1990; Nadel & Luck, 1992). In many cases, hosts suitable for oviposition may serve as food (Jervis & Kidd, 1986; Heimpel & Rosenheim, 1995) and thus feeding and oviposition sites are spatially coincident. In aphid parasitoids, both aphid honeydew and the aphid sex pheromone may serve as kairomones for both sexes (Hardie *et al.,* 1994; McNeil & Brodeur, 1995; Powell *et al.,* 1998).

- Acoustic signals can also give precise information on mate location in insects (van den Assem & Putters, 1980). The typical example of male grasshoppers that produce a stridulatory signal to attract receptive females is well known. The acoustic signal used in insect mate-finding communication could also be water vibrations, leaf shaking, or substrate thumping (recent review in Cocroft & Rodriguez, 2005). At a short range, wing vibrations can be used during mating (Joyce *et al.,* 2008; Danci *et al.,* 2010). Recently, Villagra *et al.* (2011) reported evidence that wing fanning by *A. ervi* constitutes a courtship song.

- Visual signals are probably effective for some species over longer distances, but only during daylight hours and where there is little cover. Some species passively use their body surfaces as signal generators but others use specific movements (Thornill & Alcock, 2001). It is important to take into account bioluminescent signals used by some insects to locate and choose their mate (Lloyd, 1983 for a review). In parasitoids, visuals cues used by females are known (Battaglia *et al.,* 2000) but no evidence for the use of visual signals during mating has been observed.

Courtship

The courtship phase is defined as the part of the mating sequence prior to copulation, when both sexes respond to one another. Watson & Lighton (1994) included two steps in courtship: decreasing the distance between sexes and influencing female choice (persuasive courtship).

In insects, a confrontation with females is not always necessary to induce male courtship and mating behaviour. Dummy females can be used to investigate which cues males respond to in courtship and a crude dummy can be sufficient provided it carries the correct chemical cues (Battaglia *et al.,* 2002). Male display position is mainly stereotyped but may differ between taxa (Gordh & DeBach, 1976). Once a male has assumed the correct orientation relative to the female and the female has become immobile, the male begins courtship. At a short-range, males can use acoustic stimuli to motivate the female to mate (Kimani & Overholt, 1995; Joyce *et al.,* 2008; review in Cocroft & Rodriguez, 2005). In parasitoid wasps, many males also vibrate their wings, both upon approaching the female and during the courtship (Joyce *et al.,* 2008; Villagra *et al*, 2011; Bourdais *et al.,* 2012). Chemical stimuli also play an important role in short distance communication between males and females (Casas & Magal, 2006; Danci *et al*., 2010). Contact pheromones present on the cuticle are widespread and play a role in the recognition of mating partners and in recognition of mate quality (Carazo *et al.,* 2004; McClure *et al.,* 2007; Ruther *et al.,* 2009).

Timing of mating: the mating window

We define here the mating window as "*the time interval during which an individual accepts to mate*". This period begins with the sexual maturation and finishes with the moment when the individual is unreceptive. The mating window can be different for males and females in the same species, for instance when one sex needs a longer sexual maturation period than the other. In parasitoids species, few data exist on sexual maturation period in both sexes.

Some **female** parasitoids are not immediately receptive to males after emergence. This could have evolved to avoid inbreeding. If siblings emerge near each other, males and female are likely to be related. Thus, a post emergence refractory period may lessen the risk of mating between siblings without any need of a behavioural avoidance (Antolin & Strand, 1992). In some parasitoids, when females fail to find a mate early in life, they are subsequently unreceptive to male courtship and thus produce only males. Older studies on Braconidae species found that females are reluctant to mating after one day (Drea *et al.,* 1972; Wiackowski, 1962). In aphid parasitoids, He *et al.* (2004) showed that *A. ervi* females do not need a sexual maturation period but that males needed 4 hours to be sexually mature.

The mating window in **males** is even less studied than in females. Males of virtually all species are supposed to mate more or less immediately after emergence but, in practice, the reality is less clear. In *Trichogramma sp.* males do not seem to need a sexual maturation to be chosen by females (Doyon & Boivin, 2006). This could be linked with their on-patch mating strategy just after emergence. In *B. hebetor*, males and females are unreceptive to mating during the first 2 hours after emergence, which helps to avoid sibmating on the natal patch (Ode *et al.,* 1995). *Fopius arisanus* Sonan (Hymenoptera, Braconidae) is an exception in parasitoids because males will copulate only after a prematuration period of several days post emergence due to sperm movements in the male genital tract (Quimio & Walter, 2000).

Copulation and insemination

All winged insects actually copulate (Alexander, 1964), although Odonata males must first load sperm into an organ separated from the opening of the genital tract (Corbet, 1962) before copulation. Copulation duration can depend on female body size, but is less despendent on male body size (Lefranc & Bungaard, 2000 and references therein). In several species, receptive females remain in the copulation posture for similar

periods irrespective of whether copulation has occurred (van den Assem *et al.,* 1980; pers. obs. in *A. matricariae*).

In some male insects, spermatozoa are produced in the testes during late larval and pupal stages, and the adult male emerges with a more or less complete sperm stock (prospermagenic males, Boivin *et al*., 2005). In others, adult males produce viable sperm during their life (synspermatogenic males, Boivin *et al*., 2005). When the male does not encounter mates for a long time, sperm storage structures maintain the virility of spermatozoa (Damiens *et al*., 2003). In general, males are able to inseminate more than one female. However, males that copulate with many females in a rapid succession may temporally deplete their supply of sperm (Laing & Caltagirone, 1969; Damiens & Boivin, 2006). Recently, it has been shown that males can adjust the ejaculate size in function of various parameters such as female mating status or female body size (Pitnick & Markow, 1994; Damiens & Boivin, 2005; Martel *et al*., 2008a).

Male ejaculates contain numerous substances other than spermatozoa that have diverse physiological and behavioural effects on females: reducing the likelihood of re-mating, stimulation of egg production and oviposition, initiating sperm storage and/or release and shortening lifespan (Chapman, 2001; Wolfner *et al.,* 2007; Chapman, 2008). A recent meta-analysis from South & Lewis (2010) showed that male ejaculates could produce opposite effects on different female fitness components (longevity, fecundity, etc…). Across most arthropod taxa, females show a significantly higher fecundity (both partial and lifetime) after receiving more male ejaculate. However, greater ejaculate quantity had the opposite effect on female lifespan, particularly for Diptera and Lepidoptera (South & Lewis, 2010).

Number of mate of each sex

For insect females, reproductive success is determined more by the number of offspring produced than by the number of matings (Bateman, 1948). Females of many species are known to mate multiply although the act of mating involvs numerous costs such as time and energy loss (Thornill & Alcock, 2001), risk of predation (Arnqvist, 1989), risk of pathogen transmission (Hurst *et al.,* 1995), physical injury (Crudgington & Siva-Jothy, 2000), and toxic effects of substances from male ejaculates (Chapman *et al.,* 1995). On the other hand, multiple matings may be advantageous for females because they increase sperm supplies and could stimulate egg production. Arnqvist & Nilsson (2000) showed that many insect species seemed to benefit from polyandry, but their study was clearly biased towards polyandrous species because most research addressing the direct benefits of polyandry had been carried on polyandrous species.

Mating frequencies among parasitoids has been well studied in Hymenoptera. Ridley (1993) reviewed the literature and showed that around 80% of female parasitoid wasps only mated once. Females of most monoandrous parasitoid species are not receptive to further courtship after successful insemination, although there is much variation among species. Females of some species ignore males after insemination, whereas some are just less receptive but may succumb to prolonged courtship (Van den Assem & Visser, 1976; Khan *et al.,* 2005) or an experimentally modified mating sequence (Arnqvist & Andres, 2006). Ridley (1993) also showed that there is a significant tendency for gregarious species to mate several times and for solitary species to be monoandrous. However, few studies demonstrate the polyandrous or monoandrous state of a female. As multiple matings occur rapidly and are easily observable, it is easy to prove polyandry. On the contrary, to ensure that a species is monoandrous is difficult. In Aphidiinae, old studies reported that *Aphidius* females are monoandrous (Ridley, 1993 and references therein), but more recent studies suggest that repeated mating can occur under lab conditions (McNeil & Brodeur, 1995; McClure *et al.,* 2007).

The number of times that males mate is a less studied parameter because most males are able to mate with numerous females in insect species (Thornill & Alcock, 2001). We have indications on the number of mates in recent studies about sperm allocation in parasitoids (review in Boivin *et al.,* 2005). In parasitoids species, males are supposed to be polygynous and the lifetime mating potential of hymenopteran parasitoids males varies from 3 to 56 females (Henter, 2004). Males of *B. hebetor* Say (Hymenoptera, Braconidae) can inseminate up to 50 females (Ode *et al.,* 1996). Males of *N. vitripennis* Walker (Hymenoptera, Pteromalidae) can inseminate up to 30 females (van den Assem, 1986; Reece *et al.,* 2004). What complicates the counting of the number of matings performed by a male is the fact that some males continue to mate even when sperm is depleted (Jacob & Boivin, 2004; Damiens & Boivin, 2005). For instance, males *Trichogramma turkestanica* are able to inseminate 10 females and then partially inseminate 10 more (Damiens & Boivin, 2005).

Post mating events

Sperm competition is the selective force that arises whenever the functional sperm of two or more males overlap in time in a single fertile female (Parker, 1984). Sperm competition is linked to post mating sexual selection including both male-male competition and female choice (cryptic female choice) (Danielsson, 1998).

In most insect species, sperm are prevented from travelling directly to the eggs and are shunted into one or more spermathecae that store the sperm received from copulatory partners. Except for short living species, the use of spermatheca has various benefits. The female does not need to mate prior to each egg laying period and can use sperm only when it is needed. It can free females from some costs linked with mate search or the mating behaviour itself (loose in time, energy and higher risk of predation). Third, a sperm storage organ allows the female to control the fertilization. As the egg passes down the oviduct and near the spermatheca, the female releases a controlled quantity of sperm, sufficient to fertilize the egg. In haplodiploid species, there is a special advantage of sperm storage and control of fertilization: the female can adjust the sex ratio depending of various internal or external factors by choosing to fertilize or not its eggs.

Many structural and behavioural attributes have evolved in males that can be interpreted as devices to facilitate sperm precedence (Thornill & Alcock, 2001). Evolution thus favours males that can affect the storage of other males' sperm or use their sperm in such a way that his own fertilization success is maximised. On the other hand, evolution should also favour males that are able to prevent or reduce subsequent competition from sperm of other males with some adaptations to prevent females from remating (Danielsson, 1998). The penis can be used to remove a rival's sperm, as well as to place the male's own gametes in a strategic position close to the spermatheca. It is well known in damselflies that male aedeagus scrapes and pulls out rival ejaculate from the bursa copulatrix (Cordero Rivera, *et al.* 2004). In *Drosophila melanogaster*, male injects some chemicals in the female genital tract that cause the release of any sperm stored by mimicking the chemical that the female uses when she releases sperm to fertilize its eggs (Gilbertv, 1981). The last male advantage can be achieved in ways other than the removal of stored sperm. The male can copulate many times with the same female, which may improve his fertilization success. Another tactic to prevent multiple matings of the female is to prevent a female from accepting a new partner. A male can provide the female with a substance that makes her unreceptive for a certain time (Rieamann *et al.,* 1967), or can introduce a mating plug that acts as a physical barrier to other males (examples in Diptera species: Gillies, 1956; Fowler, 1973; Polak *et al*., 2001). The male can also guard the female to prevent them from accepting a new male (Alcock, 1994).

Mate choice

In the huge majority of insect species, females are the "limiting sex" because they traditionally invest more in reproduction than males (Bateman, 1948). Females usually produce large gametes, rich in energy (exept in

female parasitoids that produce low energetic gametes, Sabri *et al.*, 2011). Males make small, motile gametes: spermatozoids. Almost all insect females can find a partner and produce some fertilized eggs. In contrast, some males can fail to persuade female to copulate with them or can be excluded from the mating process by more powerful rivals. Usually, the insect males fight to copulate and the females exercise a choice in selecting a copulatory partner (Darwin, 1871; Bateman, 1948; Andresson & Simmons, 2006).

However, variations of reproduction investment between sexes could lead to a change of the sex role in the sexual game (Andresson, 1994; Andersson & Simmons, 2006). Animals in which males show greater parental effort than females are the exception that tests the rule. In that case, the theory predicts stronger female competition for mates, more critical mate choice by males, higher variance in female than male mating success, and more pronounced female secondary sexual characteristics (Ridley, 1978; Trivers, 1972). In insect species, sexual role reversal occurs in some species (Smith, 1980; Svensson & Petersson, 1988) and only in a few species of insect parasitoids (references in King *et al.,* 2005). Male mate choice is less common than female choice, being reported in only 58 species, distributed among 11 orders, and 37 families (Bonduriansky, 2001). The criteria used by these males to select females are generally virginity, size, age, and gravid status (see Bonduriansky, 2001 for a review).

Mate choice in parasitoids can occur at any stage of the mating process, from mate attraction until post-copulation (Brown, 1999), and is adaptative because potential mates vary in their resources. In parasitoids, as in other insects, mate choice is dependent on parameters such as: mate status, size, or kinship relation. Contrary to others insect species, mate choice remains poorly investigated in parasitoids.

Despite common stereotypes, males are not always indiscriminate and eager when it comes to mating. The initial response of males to the presence of females is nearly always apparent excitement.

Afterwards, there is a clear preference for **virgin** rather than mated females (Allen *et al.,* 1994; McNeil & Brodeur, 1995; Schworer *et al.,* 1999; Carazo *et al.,* 2003; Wedell *et al.,* 2002; King *et al.,* 2005; Martel *et al.,* 2008). Male mate choice in relation to the female status is often mediated by chemicals cues (sexual pheromones) (e.g. Carazo *et al.,* 2003). In *Spalangia endius* (Hymenoptera, Pteromalidae), mate choice tests were done on both sexes (King e*t al.,* 2005), which is uncommon (Bonduriansky, 2001). In this species, both sexes prefer virgin partners if given the choice.

Influence of **adult size** on mate choice is less studied but it seems that parasitoids prefer larger mates (Eggleton, 1990; Henter, 2004; Joyce *et al.,* 2009), probably because male and female parasitoid size may be heritable (Ellers *et al.,* 2001), although it can also be influenced by other

factors (e.g. host size; Joyce *et al.,* 2002). Size is often correlated in parasitoids with longevity (Ode *et al.,* 1996), quantity of spermatozoids (Lacoume *et al.,* 2007), or female fecundity (Rivero & West, 2002).

Mate choice in relation to **kinship** has been poorly investigated and sib-mating avoidance seems to be dependant on the species (Ode *et al.,* 1995; Gu & Dorn, 2003; Reece *et al.,* 2004; Abe *et al.,* 2005; Martel *et al.,* 2008). In relation to the mating system and the sex determination mechanism of *Bracon hebetor*, Ode *et al.* (1995) found that females tend to avoid mating with brood mates when given a choice between two males. A study on *Nasonia vitripennis* shows that females did not seem to discriminate between sibs (Reece *et al.,* 2004) and the same phenomenon is reported in *Cotesia glomerata* (Gu & Dorn, 2003).

2.3.5 Modes of reproduction and consequences on the evolution of mating system.

Modes of reproduction in parasitoids

Reproduction is the biological process by which "offspring" individuals are produced by their "parents". Reproduction is a fundamental feature of all known life; each individual organism exists as the result of reproduction. The known methods of reproduction are broadly grouped into two main types: sexual and asexual.

Sexual reproduction is the production of offspring that differ genetically from the parent. Typically, sex is thus synonymous with meiosis. Meiosis helps guarantee that an individual's progeny will differ genetically from the parent because of two points: the chromosome number of the gamete is reduced by a half and, afterward, there may be recombination or exchange of genetic material between homologous chromosomes. In most cases, each of the two parent organisms contributes half of the offspring's genetic makeup by creating haploid gametes via meiosis. The vast majority of insect species reproduces sexually and uses meiosis to produce haploid gametes: males producing sperm and females eggs.

Sex determination in Hymenoptera

One very interesting case of reproduction mode in insects is **arrhenotokous parthenogenesis,** that derives from sexual parthenogenesis. Arrhenotoky occurs in around 20% of animals, such as ticks and mites (Acarina), scale insects (Coccoidea, Margarodidae), or thrips (Thysanoptera) and characterizes the entire insect order Hymenoptera, which contains more

than 200 000 species (Bull, 1981; 1983; Boivin *et al*., 2012), including many parasitoids.

Under arrhenotoky, fertilized eggs generally develop into female offspring and unfertilized eggs develop into males. This is called haplo-diploidy. Once mated, females store sperm and potentially control the sex of offspring by regulating the fertilization of eggs. This mode of reproduction induces a sex determination different than in diplo-diploid species.

Sex determination in hymenoptera species cannot be explained by heteromorphic sex chromosomes and several models have been proposed as possible explanations:

- The complementary sex determination (CSD) has been shown to fit with some arrhenotokous parthenogenetic Hymenoptera. In this model, the sex is determined by one (Single Locus Complementary Sex Determination, sl-CSD) or more (Multiple Loci Complementary Sex Determination, ml-CSD) sex loci.

Females are heterozygous while normal males are haploid and have just one allele. Individuals that are diploid and homozygous develop into diploid males. The number of allele per loci varies between species but is often high (9 for *Bracon hebetor*, Whiting, 1943; 19 for *Apis mellifera,* Adam *et al.,* 1977).

In this model of sex determination, **inbreeding** has a great impact as it increases homozygosity, and therefore diploid male production. For instance, the diploid offspring of any mother/son mating should be 50% male in case of sl-CSD. Under panmixis, the frequency of matched matings is $2/k$, where k is the number of sex alleles in a population. As half of the diploid offspring from a matched mating develop into males, the frequency of a diploid being male is $1/k$ under random mating (Cook & Crozier, 1995). Under inbreeding, the likelihood of matched matings rises and so does the occurrence of diploid males (Cook & Crozier, 1995). When siblings are the offspring of unmatched parents, the probability of a sibling mating being matched is 0.5, regardless of k. Hence, the expected proportion of diploids developing into males is at least 0.25 under inbreeding (figure 3).

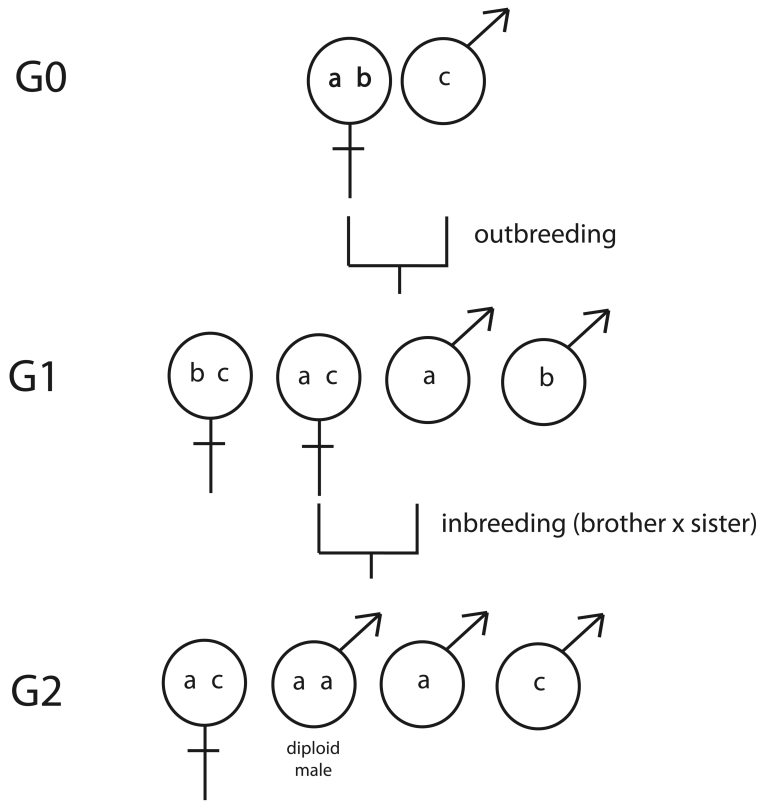

Figure 3 Consequences of sl-CSD on the offspring in the case of outbred and inbred matings. Diploid individuals that are homozygous are males.

Diploid males are commonly assumed to carry a genetic load for a population. They are produced at the expense of females, thus skewing the sex ratio towards males and potentially inhibiting the growth of populations and jeopardizing their persistence (Godfray, 1994; Zayed & Packer, 2005). Additionally, diploid males often suffer from low viability or are effectively sterile (Cook, 1993; de Boer *et al*., 2007).

Since Whiting's discovery in 1943, CSD has been found in over 60 species of Hymenoptera (van Wilgenburg *et al.,* 2006), including several economically important species, such as honeybees, fire ants, and numerous parasitoids. CSD is considered ancestral although few species from the basal taxonomic groups have been tested for CSD (figure 4).

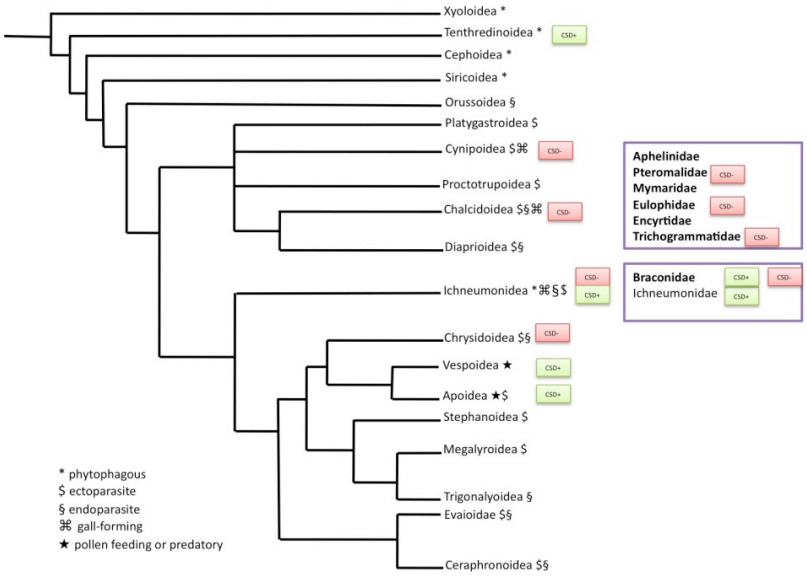

Figure 4. Phylogeny of Hymenoptera and relation with the CDS model (after Cook, 1993; Heimpel & De Boer, 2008; van Wilgenburg *et al*. ,2006) CSD- signifies that Complementary Sex Determination is absent in the taxa and CSD+ signifies that it is present in at least one species.

Reviews of CSD have focused on general aspects such as the distribution of species with and without CSD (Cook, 1993a; van Wilgenburg *et al.,* 2006), population-level and evolutionary consequences of CSD (Stouthamer *et al.,* 1992; Cook & Crozier, 1995; van Wilgenburg *et al.,* 2006), the implications of CSD for Hymenoptera mating systems (Godfray & Cook, 1997), and the initial discoveries concerning the molecular basis of CSD (Page *et al.,* 2002; Beye, 2004; Charlesworth, 2004; Evans *et al.,* 2004).

The implications of CSD for the biological control of insect pests have also been discussed (Stouthamer *et al.,* 1992; Heimpel & Lundgren, 2000; Wu *et al.,* 2003; Salin *et al.,* 2004; Ode & Hardy, 2007). Indeed, biological control relies on release of mass-reared control agents. Many of these control agents have single locus CSD (Wu *et al.,* 2003). Therefore, mass-reared populations, if they are isolated in a lab, should lead to deleterious consequences. Species with fertile diploid males should, therefore, have better prospects of success as biological control agents (Cowan *et al.,* 2004; Elias *et al.,* 2009).

- The genomic imprinting sex determination (GISD) model (Poiré *et al.,* 1993; Beukeboom, 1995; Dobson & Tanouye, 1998) proposes that one or more loci are differentially imprinted in paternal *versus* maternal development. Unfertilized embryos contain only maternally derived autosomes with the "maternal imprint," and result in males. Fertilized embryos contain autosomes with the "paternal imprint" in addition to maternally derived autosomes, and the resulting individual develops as a female. Evidence for this kind of sex determination was found in *Nasonia vitripennis* (Dobson & Tanouye, 1998).

- The genic balance sex determination (GBSD) was proposed as an alternative way of sex determination in Hymenoptera. Although the notion of genic balance was formulated from studies of *Drosophila melanogaster*, a diplodiploid species, Bridges (1939) considered that it should also apply to the haplodiploid Hymenoptera. Under GBSD, sex is determined by female determining factors with cumulative effects and male determining factors with no or only slightly cumulative effects. In haploids, the male tendency predominates while in diploid female tendencies are cumulative and the balance become female (Da Cunha & Kerr, 1957; Keer & Nielsen, 1967). However, the accumulation of evidence for diploid males and single-locus CSD in several disparate species contradicts the genic balance theory.

Of these 4 models, single locus CSD is thought to have the biggest impact on mating systems because its effects are exacerbated with inbreeding.

Inbreeding in Hymenoptera parasitoids

Mating systems in haplodiploid parasitoids range from outbreeding to facultative inbreeding and obligate inbreeding (Godfray, 1994; Hardy, 1994; Godfray & Cook, 1997).

Previously we reported that a single sex-determining locus (Whiting, 1943, figure 4) is the ancestral mode of sex determination for the hymenoptera. However, the single locus Complementary Sex Determination is very costly and should not be present in species that mate non-randomly, i.e., at a local scale.

Mating in gregarious parasitoid species (*i.e*. two or more offspring emerging from one host) generally occurs among individuals emerging from a single host before the females disperse. Gregariousness may therefore be in conflict with sl-CSD. The three *Bracon* species seem to violate this prediction. In *B. hebetor*, sex ratios have been reported to be female-biased (Antolin & Strand, 1992). Nevertheless, sib mating in *B. hebetor* is rare for a

number of reasons. Females exhibit a pre-mating refractory period during which dispersal takes place (Ode *et al*., 1996). They have a mating preference for males that emerge from a different host (Ode *et al.,* 1996), and males aggregate in leks that attract sperm-depleted females. A pre-mating refractory period of 4 to 5 hours after emergence has also been found in *B. brevicornis* (Sudheendrakumar *et al.,* 1978*)*. In *Cotesia glomerata*, 50 to 100% of the females and 30% of the males disperse immediately after emergence from their natal patch. This results in only a minority of 25% of females mating with sib males in the field (Gu & Dorn, 2003). Clearly, these behaviours promote an outcrossing mating system.

Other species of parasitoids do not seem sensitive to inbreeding. For example, *Nasonia vitripennis*, *Trichogramma sp.* or *Melittobia sp*. mate on their natal patch, usually with their sibs and do not have a sl-CSD (Godfray & Cook, 1997; Hardy *et al.,* 2005; van Wilgenburg *et al.,* 2006).

2.4. Parasitoids mating systems: What is still missing?

There is an increasing amount of data about parasitoid physiology and behaviour with many descriptive results about their mating strategies (see some recent book reviews: Hochberg & Ives, 2000; Jervis, 2005; Wajnberg *et al.,* 2008). However, even for some of the most studied model species (*Trichogramma sp.*, *Aphidius sp.*, *N. vitripennis*), there remain some missing important points for the correct interpretation of their mating system (sex-ratio, mate choice aspects, mating window…). For the huge majority of species, females have received much more attention than males, however recent studies on male mate choice and ejaculation strategies had been performed (van den Assem, 1986; Bondruansky, 2001; Villagra *et al.,* 2005; Damiens *et al*., 2006; etc). Male and female investment in reproduction is probably not so different and both sexes are supposed to optimize their mate choice to increase their own fitness. Mutual mate choice has received attention in other taxa and should be explored in parasitoids, mainly because some are sensitive to inbreeding.

The link between mating strategies, sex determination, and phylogeny of parasitoids is still missing in the literature, despite of its importance to understand strategies that have evolved in these species, as explained before. Moreover, we observe that some life history traits (e.g. sexual maturation, pattern of emergence, number of mates) are different between closely related species and have to be taken into account. One relevant example is the *Cotesia* case. Two *Cotesia* species behave differently

in mating because of their resistance to inbreeding, linked with sex determination (Gu & Dorn, 2003).

We probably need a more comparative approach of parasitoid mating systems that compiles the descriptive data of the literature with sex determination rules and resistance to inbreeding. The recent phylogenetic model of Hymenoptera and Braconidae taxa could help us to have a more integrative view of parasitoid mating strategies than the current model.

Moreover, within parasitoid species, we need more data about koinobiont species that are quasi-gregarious, especially aphid parasitoids. In these species, the host is still alive some days after parasitism and can change its behaviour. Though it has been known for many years that aphids change their behaviour after the attack of the colony by Aphidiinae wasps, there are still some missing points and a significant "black-box" between the moment of the attack and the moment of emergence of new adults for most of the host-parasitoid complexes. In particular, we have no data about the emergence pattern in space of aphid parasitoids, and we do not know if they really emerge as quasi-gregarious species. From a fundamental point of view, various studies have not allowed us to state whether the behaviour is favourable to the host or to the parasitoid or both. We know that parasitoids can parasitize alate aphids, but few studies take this in account to evaluate how this could influence gene flow between parasitoids populations.

3. The special case of aphid parasitoids

3.1. Aphid parasitoids: who are they?

3.1.1. Diversity

Primary parasitoids of aphids are found in two taxa, the Braconidae and the Aphelinidae. These two groups differ in several traits, especially those associated with reproduction. In Aphidiinae, very closely related species range from host specific to host generalist (e.g. *A. matricariae vs. A. rhopalosiphi*). Aphidiinae is a monophyletic subfamily of Braconidae that comprises over 400 species and 50 genera, divided in 4 groups (figure 5, Smith & Kambhampati 2000). Aphelinidae contain few genera that

parasitize aphids: *Aphelinus*, *Marietta*, *Protaphelinus* and *Mesidiopsis* (Boivin *et al*., 2012, figure 4). Moreover, some species belonging to Cecidomyiidae (Diptera) were described as being endoparasitoids of aphids (Muratori *et al.,* 2009).

Aphid parasitoids are **koinobiont** species, which means that the aphid stays alive during parasitoid larval development and is still able to disperse from its natal colony. The larvae develop as a solitary endoparasitoid, feeding selectively on its host's tissues and organs (Sabri *et al.,* 2011). The last larval instar kills the aphid and pupates either inside or in a separate cocoon below the mummified host (Sabri *et al*., 2011).

3.1.2. Aphid parasitoid mating system: state of the art

A recent review of Le Ralec *et al*. (2010) explained the evolutionary ecology of the interactions betweens aphids and their parasitoids. They review a large panel of traits (aphid behavioural defence towards parasitoids, host exploitation strategies, diapause, and consequences of climate change…) but unfortunately do not explain how the mating strategies of the parasitoid could be linked to its host's behaviour and ecological patterns. The same lack of data was present in the review of Boivin *et al.* (2012) on the use of aphid parasitoids in biological control. Figure 6 reviews the current knowledge of various aspects of mating strategies of aphid parasitoids.

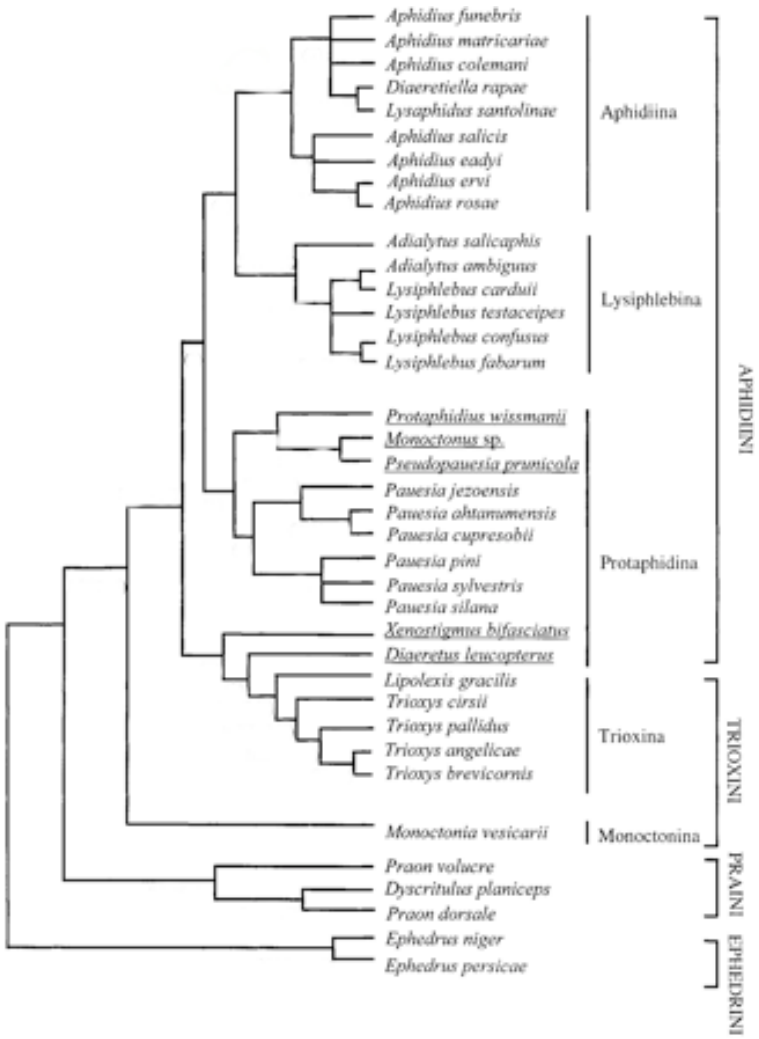

Figure 5. Phylogeny of Aphidiinae sub-familly (after Sanchis *et al.* 2000)

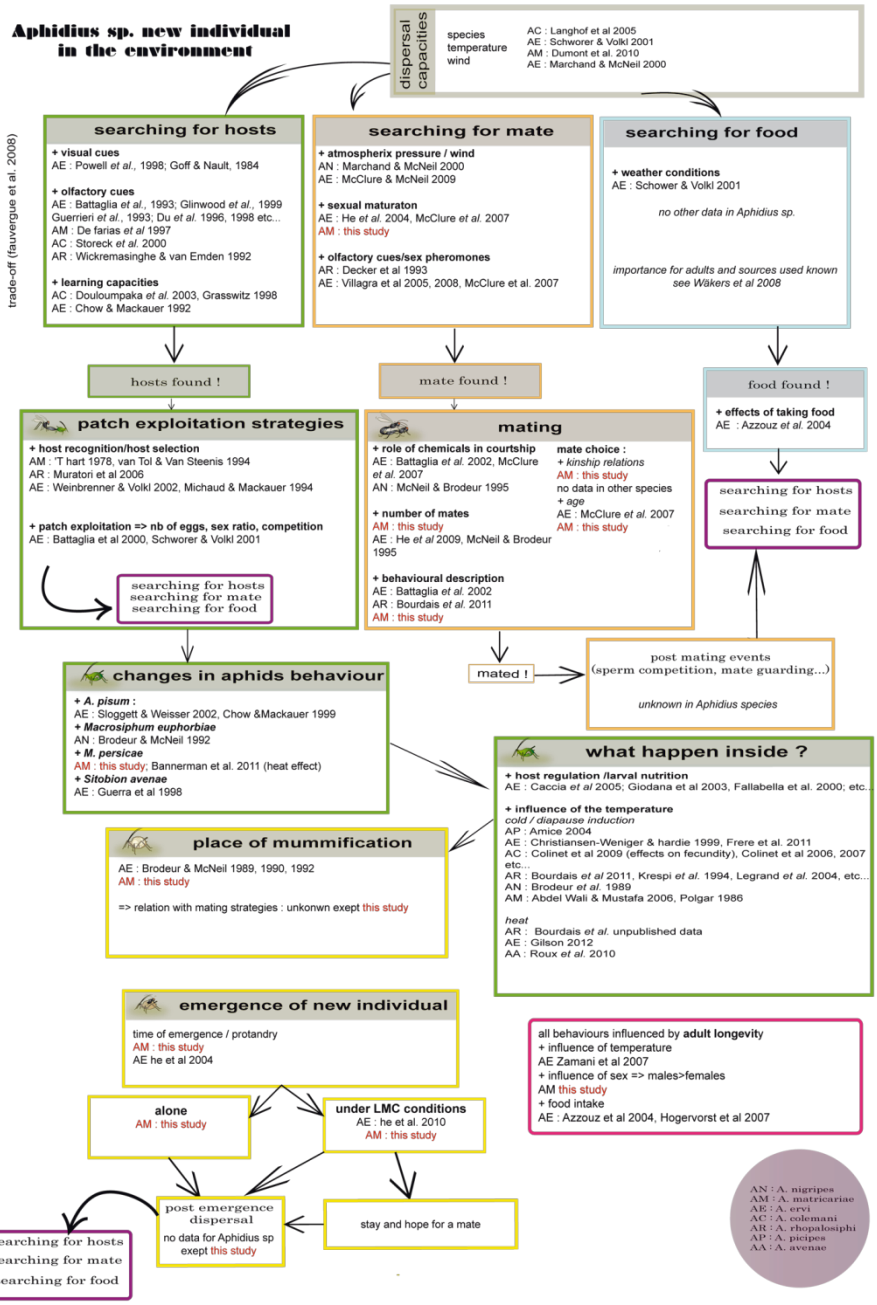

Figure 6. Diagram showing a non-exhaustive list of past studies on *Aphidius sp* parasitoids and data collected during the present study.

Physiological and behavioural aspects of mating

Sex pheromones are well known in aphid parasitoids (Decker *et al*., 1993; Reed *et al*., 1994; Bodeur & McNeil, 1995; McNeil *et al*., 2007). In the presence of a virgin female, the first evident sign of short-distance male response to pheromones is a series of wing vibration bouts and an increase in general locomotor activity (Bodeur & McNeil, 1995; Bourdais *et al.,* 2012). In *A. nigripes*, the site of pheromone production appears to be located in the abdomen (Brodeur & McNeil, 1995). The evolution of female pheromone production suggests that it may decrease with age, explaining why old females are less attractive (Brodeur & Mc Neil, 1995).

It is clear from direct field observations that males **exhibit upwind flight** towards virgin females placed in sticky traps (Bodeur & McNeil, 1995). Moreover, the field results clearly demonstrated a **diel periodicity** with a peak early in the morning that coincides with the time that they observe most the matings in the laboratory (Brodeur & McNeil, 1995). Reed *et al.* (1994) also reported that most of *A. colemani* found in female-baited traps were captured before noon.

Male *Aphidius ervi* (Hymenoptera: Braconidae) can **learn to associate** and respondto an artificial odour (vanilla) with a sexual display, after having been trained to associate the vanilla odour with the encounter of a conspecific female (Villagra *et al*., 2005). In training experiments, Villagra *et al.* (2008) show that plant odours triggered strong attraction and wing fanning courtship behaviour in trained responses when the male was exposed to a female and these odours during training. Hence, through learning, the olfactory stimulus context present during copulation could become a predictive cue for further mate searching.

In aphid parasitoids, females are supposed to be **monoandrous** (Verai, 1942; Giri *et al*., 1982; Ridley, 1993; Mc Clure *et al.,* 2007; Mc Neil & Brodeur, 1995; Kant *et al.,* 2012). However, recent studies suggest that in particular laboratory conditions, females of some species could mate twice (McNeil & Brodeur, 1995; McClure *et al.,* 2007; He & Wang, 2008). We have no data on the number of male matings, but personal observations conclud that they are able to mate with several females.

Females of *A. ervi* do not need a **sexual maturation time**, while males need at least 2 hours to be able to mate (He *et al.,* 2004). No precise evidence of the mating window of aphid parasitoids is known, as authors often use 24h old individuals for experiments (McNeil & Brodeur, 1995; Bourdais *et al.,* 2012; Battaglia *et al*., 2002; Takada & Tada, 2000; Giri *et al.,* 1982; Verai, 1942; Cloutier *et al.,* 2000, etc…).

The influence of **mating status on foraging behaviour** of females has been examined in different species (Michaud, 1994). Virgin females abandoned bean shoots infested with pea aphid earlier than their mated counterparts (Michaud, 1994). Virgin females attacked fewer aphids within a patch than mated females, and parasitized fewer hosts in a 2-h period. Females therefore tend to produce a smaller brood within a patch when unable to fertilize their eggs and produce daughters (Michaud, 1994)

Examination of females captured in the field showed that virtually **all were mated** (Mackauer, 1976; Mishra & Singh, 1991), with only a small proportion being constrained and capable of producing only male offspring. A female may be constrained because she has found no suitable mating partner; however, even a mated female may be constrained during a variable period after insemination when sperm is unavailable (Mackauer, 1976; van den Assem, 1977).

Aphidius parasitoids exhibit some **mate choice**, in that mating success has been shown to be the greatest when individuals are from the same host species population (Powell & Wright, 1988; Henry, 2008).

In species with **low resource utilization per patch**, such as many *Aphidius* species (Mackauer & Völkl, 1993; Weisser, 1995; Schwörer & Völkl, 2001), the number of potential mates emerging simultaneously is generally lower than in species with high resource utilization, such as *L. hirticornis* (Völkl, 1994; Weisser, 2000). Therefore, the probability of sib-mating is expected to be lower in species producing only few offspring per patch. Schwörer & Völkl (2001) suggested that *A. ervi* was a "low resource user" based on results of manipulation experiments in which females attacked less than one third of suitable *A. pisum* hosts. Field observations on *A. ervi* have documented female-biased sex ratios (Sequeira & Mackauer, 1993). According to Scwörer & Volkl (2001), aphid parasitoid mating systems could be characterized by dispersal from the natal patch and outbreeding. This is typical of species parasitizing only one or few aphids in each colony such as *A. ervi* (Schwörer & Volkl, 2001). In comparison, the other kind of mating system is characterized by a high degree of sib-mating and LMC on the natal patch; it is typical of species producing large clutches such as *L. hirticornis*. The two systems are not mutually exclusive but represent the extremes of a continuum; the position of each species on this continuum depends on its pattern of resource utilization and average clutch size. To date, it is the only study dealing with this aspect of mating systems in aphid parasitoids.

Sex determination / inbreeding / LMC

Some evidence of single-locus Complementary Sex Determination was suggested in *Aphidius* species (*A. rhopalosiphi*, Salin *et al*., 2004) where authors found an important increase in the proportion of males with inbreeding. Further investigations have to be conducted on the Aphidiinae subfamily, especially because some Braconidae species do not share the CSD sex determination model (Beukeboom *et al.,* 2000; figure 7).

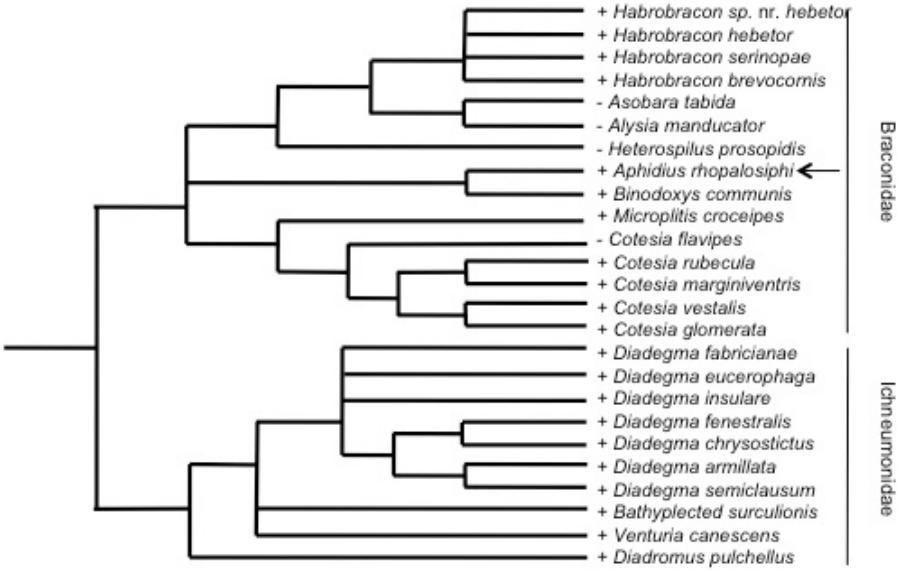

Figure 7. Most parsimonious character mapping of CSD presence (+) or absence (-) on a composite phylogeny of the Ichneumonoidea. Vertical bars delineate taxonomic families. Modified after Asplen *et al.* 2009

Sex ratios of some aphid parasitoids are reported from field experiments or commercial samples. In natural populations of *A. ervi*, the overall offspring sex ratio is significantly female-biased with a seasonal mean of 65.7% females (Sequerira & MacKauer, 1993). This value is remarkably similar to the sex ratio reported by Mackauer (1976) for field populations of other species of pea-aphid parasitoids, including *Aphidius pisivorus* 55.4% *and A. smithi* Sharma & Subba Rao (55.9%).

Sex ratios of commercially distributed aphid parasitoids are more diverse, ranging from 85% of females (*A. ervi*) to 48% in *A. colemani* (Heimpel & Lundgren, 2000).

Dispersal

Studies on *Aphidius* species have shown that dispersed females were caught on glue traps 10m around the release spot under natural conditions (Langhof *et al*., 2000; Muratori *et al.,* 2000; Dumont *et al.,* 2011). Weisser & Wolkl (1997) observed that the aphid parasitoid *Lysiphlebus cardui* can move no more than 20m when searching for hosts. In a flight chamber, flight duration is influenced by the gender and mating status of *E. eremicus* (Bellamy & Byrne, 2001). Females flew for 10.6 times longer than males and unmated parasitoids flew for 2.9 times longer than mated parasitoids. It is suspected that this behaviour is driven by a search for resources. In the laboratory, unmated parasitoids might be inclined to engage in relatively long flights because they would normally be searching for potential mates. Female flight (both mated and unmated) might also have been longer because their flight duration may reflect a search for both mates and hosts (Papaj, 2000).

The parasitoid dispersal as larvae through the parasitized aphid results from changes in the parasitized aphid behaviour or through natural flight dispersal of alate aphids.

A new method of investigating aphid parasitoid dispersal is through the study of dispersal via aphid flights. Aphid dispersal by active flight over local vegetation or passive flight with wind for long distances is a well-evolved life strategy that aphids rely upon for location of suitable plants (Dixon & Laird, 1967; Robert, 1987). Intriguingly, little effort has been made to demonstrate possible dispersal of parasitoids with host flight, although *Aphidius* species are closely associated with their hosts. Aphid parasitoids are known to parasitize alates (Pons & Stary, 2003; pers. obs. in field or our lab rearing, figure 8). These parasitized alates can fly with the larvae inside only if the larvae are small (Rauwald & Ives, 2001). However, the potential role of host dispersal flight in aphid-parasitoid interactions was only observed in recent field studies. Feng *et al.* (2007) observed in field captures 5% of alates were parasitized by two parasitoid species. The study of Pons & Stary (2003) reported the same results and alate aphids of *M. dirhodum*, *R. padi* and *S. avenae* showed a highly variable proportion of parasitism.

Figure 8. Mummy stage of an alate aphid (*Myzus persicae)* parasitized by *A. matricariae*. We can observe that the wings of the alate aphid are present in the mummy, suggesting that alate aphids can fly even once they are parasitized. ©D.Bourdais

3.2 Aphid biology

Aphids form a monophyletic group that appeared around 280 million years ago (Grimaldi & Engels, 2005). Aphids are part of the Superfamily Aphidoidae and are described as small (1-10 mm) soft-bodied insects with, or more commonly without, wings. They possess a proboscis, which originates between and behind the forelegs. Their antennae have two thick basal segments and a flagellum composed of up to 4 segments. They possess two compound eyes and two ocular tubercles made up of three lenses each that are situated behind and above the compound eyes. They have two tarsal segments. The wings, when present, have only one prominent longitudinal vein. The fifth abdominal segment bears a pair of upward and backwardly pointing tubes on the dorsal surface called siphunculi (cornicles) and a cauda is usually present below and between them on the last abdominal segment.

There are about 4 500 species of aphids in the world of which about 250 are serious pests. Aphids have a worldwide distribution but there are far more species in temperate zones (Blackman & Eastop, 2000). They cause damage as their feeding reduces the vitality of the crops they feed on and as some of them transmit viral diseases. Aphids feed on the phloem of plants with the stylets of their proboscis. Plant phloem saps are rich in sugars and poor in amino-acids or nitrogen. Therefore, as aphids must drink high amount of sap to obtain enough nitrogen, they have far more sugar and liquid than they need in their diet and they eliminate that excess by excreting large amounts of sugary liquid (the honeydew). This honeydew can often be seen on the lower leaves of infested trees on which it falls, giving them a sticky coating. Honeydew can be a substrate for the proliferation of some fungi. The spores of the fungi are carried by wind and stick to honeydew covered leaves. These spores germinate and release fungal strands that discolour the area. Moreover, leaves that are covered with fungi do not have access to adequate sunlight. This reduces the amount of food the plant can produce. In severe cases of honeydew and fungus covering, the plant will die (Blackman & Eastop, 2000).

Reproduction

The typical aphid reproductive mode indeed involves a succession of usually numerous parthenogenetic generations, followed by a single sexual generation within the annual life cycle (Simon *et al.,* 2002). The asexual phase of aphids occurs during the growing season (spring and summer) and up to 20 asexual generations can be produced if climatic conditions are favourable.

The **parthenogenetic individuals** have short developmental times and potentially high rates of increase in spring and summer. In some species, parthenogenetic individuals can produce winged forms that can disperse from the host plant to another. The components of the environment triggering the change from the unwinged to winged forms and from asexual to sexual individuals have been studied since the 1950's (Lees, 1959; Lees, 1963; Lees, 1967; Kunert *et al.,* 2005; Braendle *et al.,* 2005; 2006). Sometimes, the appearance of winged forms can be due to overcrowding and repeated tactile stimulations (Lees, 1967). Other species switch in development because of changes in the quality of the host plant (Forrest, 1970; Dixon, 1971) or because of an increasing production of alarm pheromone (Kunert *et al.,* 2005).

The change from asexual to sexual reproduction is often induced by short day length, low temperature, or quality of the host plant and is often under hormonal control (Forrest, 1970; Le Trionnaire *et al.,* 2009). Each morph has a particular role to perform and is part of a sequence, which ends with the egg laying forms (Simon *et al.,* 2002; Simon *et al.,* 2010).

Population dynamics within a year

Most aphids are autoecious (living on one or few species of closely related plants). About 10% are heteroecious spending autumn, winter, and spring on one plant species (its primary host) and summer on a different unrelated plant (its secondary host). For example, the Rosy Apple Aphid *Dysaphis plantaginae* has *Malus sp*. as its primary host and *Plantago lanceolata* as its secondary or summer host. Some heteroecious aphids such as *Myzus persicae* and *Aphis fabae* have a wide range of secondary hosts, but this is relatively rare. Most heteroecious aphids have just one primary and one secondary host.

Natural populations are patchily distributed in the habitat and characterized by frequent and rapid fluctuations in abundance (Dixon, 1998). Aphids show complex and rapidly changing dynamics within the year. Many aphids in temperate regions overwinter as eggs that can withstand a temperature of less than -20°C (Powell, 1974). The survival of the eggs and/or overwintering individuals determines the number of aphids present the following spring and often during the following year. An initial increase in population size in spring is typically followed by a steep decline during summer, and sometimes a further increase in autumn.

3.2. Aphids behavioural reactions to parasitism

Parasitized animals often differ in their behaviour from uninfected individuals (Barnard & Behnke, 1990; Horton & Moore, 1993; Poulin, 1995; Brodeur & Boivin, 2004). Behavioural changes can be induced by the parasite and may benefit the parasite by increasing its chances of survival (Brodeur & McNeil, 1989; 1990; 1992; Schmid-Hempel & Müller, 1991; Robb & Reid, 1996). It can also benefit the host by increasing its inclusive fitness (Smith Trail, 1980; McAllister & Roitberg, 1987; McAllister *et al.,* 1990). Trauma and pathology associated with parasitism can also influence behaviour (Thompson & Kavaliers, 1994; Müller *et al.,* 1997). In the latter case, the altered behaviour may not be under direct selection and could be selectively neutral. In a review of the literature, Poulin (1995) concluded that most known behavioural changes have not been demonstrated as resulting in fitness gains to either the host or parasite and may not therefore be adaptive.

In parasitoids, the host behaviour manipulation is poorly understood compared to other taxa (Bodeur & Boivin, 2004). Changes in the distribution of unparasitized and parasitized hosts are the most common behavioural manipulations reported for parasitoids (Brodeur & Boivin, 2004), especially in aphid parasitoids.

3.2.1. Aphid reactions during the attack

- Alarm pheromone: production and behavioural consequences

Aphid primary defense from predators and parasitoids consists of escape responses triggered by the release of an alarm pheromone (Pickett *et al.,* 1992; Dixon, 1998). Alarm pheromone emission occurs by secretion of a droplet of liquid from the ends of the corniculi. This method has been known for more than 35 years (Bowers *et al.,* 1972; Nault *et al.,* 1973). The major components of the secretions are triglycerides, which appear to function as a mechanical defence by gluing natural enemies (Callow *et al.,* 1973). **(E)-β-farnesene (EβF)** is the other component of the droplet of liquid excreted by the corniculi.

When an aphid is exposed to alarm pheromones, it has several behavioural choices:
1. Continue to feed without displaying any response to the pheromone. An aphid that shows no response does not pay energetic costs or sacrifice feeding opportunity. However, he does not reduce the predation risk.
2. Continue to feed but show agitation, kicking with its legs. This may prevent a small predator from carrying out a successful attack. An aphid showing agitation pays an energetic cost and somewhat reduces its predation risk.

3. Stop feeding and walk from the feeding site. An aphid walking from its feeding site pays an energetic cost for its displacement and does not feed until finding a new feeding site. It benefits from a reduction in predation risk.
4. Stop feeding and drop from the plant. An aphid dropping from the host plant must locate and climb another host plant. It risks desiccation if it cannot locate a suitable host within some minutes. Dropping behaviour is a general response to disturbance in many aphids, however, independent of the cause of disturbance, including direct attacks by foraging parasitoids and predators (Dill *et al.,* 1990; Stadler *et al.,* 1994; Chau & Mackauer, 1997; Losey & Denno, 1998). Brodsky & Barlow (1986) and Clegg & Barlow (1982) have described the escape behaviour of pea aphids in response to an alarm pheromone. McAllister & Roitberg (1987) and McAllister *et al.* (1990) interpreted the dropping behaviour of parasitized pea aphids (*Acyrthosiphon pisum* Harris) as "host suicide". Aphids parasitized by *Aphidius ervi* Haliday often dropped from their feeding site when disturbed, thus increasing their own mortality risk while reducing the risk of parasitism to other members of the clone for an overall gain in inclusive fitness.

EβF has been shown to be the primary alarm pheromone for many aphid species, including the green peach aphid *Myzus persicae* (sulzer) (Edwards *et al*., 1973; Francis *et al.,* 2005) and the pea aphid *Acyrthosiphon pisum* (Harris) (Du *et al.,* 1998; Francis *et al.,* 2005). The use of an alarm pheromone is presumed to benefit the population by allowing increased survival of related individuals taking successful evasive action after perception of the pheromone.

The **response** of aphids to the alarm pheromone can be influenced by environmental factors such as temperature. At higher temperatures, aphids are less responsive to the aphid alarm pheromone (by dropping, running, or backing up) released under attack by insect **predators** and **parasitoids** (Bayaa, 2008). Another factor that can influence aphid response to an alarm pheromone is aphid-life stage (Byers, 2005). In pea aphids, adult and fourth-instar larvae have adequate responses to alarm pheromone (by either dropping, running, or backing up), whereas younger instars showed almost no response to alarm pheromone. It was suggested that younger instars respond conservatively to alarm pheromone because they are less active on the ground and are more likely to die before finding a suitable food plant (Roitberg & Meyers, 1978). Moreover, although the alarm pheromone is used in many aphids, some species are more responsive than others (Losey & Deznno, 1998), with variation even among different clonal lines of the same species (Muller, 1983; Braendle & Weisser, 2001). It was also suggested that EβF leads to a 'pseudo crowding' effect that induces groups of aphids to produce a higher proportion of winged dispersal morphs among their offspring (Kunert *et al.,* 2005; Podjasek *et al.,* 2005). Kunert *et al.* (2005) also found that aphids react more strongly to the frequency of

pheromone release than the amount of pheromone delivered for producing winged dispersal morphs.

Alarm pheromones also can act as a **kairomonal cue** for aphid natural enemies including ladybeetles, hoverflies, and parasitic hymenoptera (Francis *et al.,* 2005; Verheggen *et al*., 2008). Although many of the long-range olfactory cues used by aphid parasitoids for host location originate from the host plant, they can also use honeydew or aphid pheromones as short-range cues (Rehman & Powell, 2010). EβF attracts *Aphidius uzbekistanicus* (Micha & Wyss, 1996), *Aphidius ervi* (Du *et al.,* 1998) or *Diaeretiella rapae* (Foster *et al*., 2005).

- Production of wax

A droplet of waxy exudate that contains triglycerides can be secreted from the glandular base of the corniculi, resulting from contraction of the muscle in the siphunculus. This droplet will rapidly solidify in the air and may play a defensive role by gumming up the cephalic parts of predator, or by reducing their feeding or oviposition (Nishino *et al*., 1976; Minks & Harrewijn, 1987; Beale *et al.,* 2006).

3.2.2. Aphid behaviour after the attack

Two non-exclusive hypotheses have been suggested to account for the evolution of behavioural changes in hosts parasitized by insect parasitoids (Stamps, 1981; Schmid-Hempel & Muller, 1991; Muller & Schmid-Hempel, 1992; Poulin, 1992).

- The first hypothesis argues that changes are t**o the host's advantage**. By changing its behaviour, the host increases its probability of dying, and reduces the parasitoid likelihood of successful development and the probability of subsequent attack of hosts' kin (Smith Trail, 1980). *A. pisum* aphids parasitized by *A. ervi* females often dropped from the host plant when disturbed, thus increasing risk of desiccation, predation, and death (McAllister & Roitberg, 1987). These results were interpreted in terms of "adaptive suicide" by parasitized aphids. It was interpreted as a kin-selected sacrifice of parasitized individuals but this interpretation has been criticized (Brodeur & Boivin, 2004; Godfray, 1994).

- The second hypothesis states that the **parasitoid modifies the behaviour of its host for its own fitness** (Fritz, 1982). Parasitoids can modify host behaviour in different ways, and parasitoid-induced behaviours are normal components of the host's behavioural repertoire. Host manipulation is needed only when requirements of the parasitoid differ from

those of the host (Brodeur & Boivin, 2004). Thus, the onset of behavioural manipulations should coincide with a period of vulnerability (Fritz, 1982), such as the pupal stage.

Many parasitoids are attacked by predators or obligate hyperparasitoids. In this case, clumped of parasitized hosts are more attractive. The primary parasitoid should be strongly selected to manipulate host behaviour to lessen the risk of hyperparasitism, especially to avoid attacks of Pteromalidae and Megaspilidae that attack the mummy stage (Mackauer & Völkl, 1993). Brodeur and McNeil (1989, 1990, 1992) reported that altered behaviour in potato aphids (*Macrosiphum euphorbiae* Thomas) was mediated by the parasitoid *Aphidius nigripes* Ashmead. Depending on the season, parasitized aphids dispersed from their feeding sites shortly before death and selected safe habitats in order to reduce the risk of hyperparasitism (Brodeur & McNeil, 1992) or to increase overwintering survival (Brodeur & McNeil, 1989; 1990). In a study on cereal aphids, Guerra *et al.* (1998) found that parasitized aphids are significantly repelled by conspecifics a few hours before mummification, suggesting that the movement of parasitized aphids away from the feeding site shortly before mummification should result from the parasitoid alteration of the aphid response to conspecifics (Guerra *et al.,* 1998).

However, both hypotheses concerning behavioural changes may be applicable, depending on the ontogenic stage of the aphid-parasitoid complex. As shown by Müller *et al*. (1997), patterns of modified aphid behaviour are highly variable, differing between species of parasitoids and between species of aphids, with both the season and the presence of trophobiotic ants. The alternative hypothesis is that the parasitized aphid dispersal is the result of the dispersal of all aphids induced by the attack of the colony by the parasitoid female. Parasitized aphids may mummify by chance on or off plants, depending on circumstances.

3.3. Consequences of aphid behaviour on its parasitoid mating system

Aphid parasitoids are traditionally considered as quasi-gregarious species because they parasitize clumped hosts (Godfray, 1994; He & Wang, 2008; Mackauer & Volkl, 2002). However, aphids disperse from their natal colony when disturbed by the foraging behaviour of the parasitoid female or by a predator attack of the colony (Pickett *et al.,* 1992; Vandermoten *et al.,* 2012). The non-clumped localisation of mummies due to aphid dispersal could have various consequences on the mating strategies of the parasitoid. One aphid colony is generally parasitized by one or few females (Schwörer & Völkl, 2001; Le Ralec *et al.,* 2010). The adults that will emerge from the

aphid of the colony will thus be sibs competing for mates. If aphids stay clumped after parasitism, aphid parasitoids will emerge under classical Local Mate Competition (Hamilton, 1967), but if aphids disperse, parasitoids will emerge less clumped in the environment. Maybe the more obvious consequence of aphid dispersal for the parasitoid is thus parasitoid mate finding. As explained before, gregarious and quasi-gregarious species usually mate just after their emergence on the natal patch. This mating system increases the probability of being mated for females but also increases the risk of sib-mating because brothers and sisters usually emerge from the same natal patch. It seems that the chance of encountering a mate out of the patch is very low (Waage & Ming, 1984). However, the proportion of virgin females is higher in gregarious species than in solitary ones (Godfray & Hardy, 1993), suggesting that solitary species have developed powerful strategies to find a mate in the environment, such as being able to respond to volatile pheromones of kairomones.

It is thus surprising that no study has analysed the relationship between aphid behaviour and parasitoid mating strategies. To our knowledge, we have no field data about the proportion of unmated females in aphid parasitoids nor the spatial distribution of mummies and emergence of adults in field conditions. However, laboratory studies on aphid parasitoids bring some evidence that some particular behavioural traits are compatible with the solitary emergence of adults that have to find a mate, and also evidence for a relationship between these behaviours and inbreeding avoidance.

Aphid parasitoids seem sensitive to inbreeding (Salin *et al.,* 2004), suggesting that either they do not emerge in real patches as quasi-gregarious parasitoids(*Trichogramma* wasps), and if they emerge in the same patch some mechanisms have been selected to avoid sib-mating (as in *Bracon hebetor*, Ode *et al.*, 1995). Males are known to respond to volatile sex pheromones (Decker *et al.,* 1993; Nazzi *et al.,* 1996; Glinwood *et al.,* 1999; Mc Neil & Brodeur, 1995; McClure *et al.,* 2007), and some studies report a male attraction to kairomones (Battaglia *et al.,* 1993) that could help them locate a virgin female if the mate out of their natal patch. Lastly, aphid parasitoids are monoandrous, which is more a characteristic of solitary species than gregarious ones (Ridley, 1993) with on-patch mating between siblings.

4. Structure of the study

Among all organisms, some species are more or less sensitive to inbreeding and have developed different strategies to avoid sib-mating. Parasitoids belong to various taxa such as Diptera, Coleoptera or Hymenoptera (Godfay, 1994). Within Hymenoptera, various mechanisms of sex determination influence the level of sensitivity to inbreeding, which may result in different adaptive traits to avoid inbreeding.

On one hand, parasitoids of the superfamilly Chalcidoidea, which include Trichogrammatidae and *Nasonia sp*., do not share the CSD mode of reproduction as in many other Hymenoptera species and are less sensitive to inbreeding depression (Heimpel & DeBoer, 2008).

On the other hand, some members of the Braconidae family share the CSD mode of reproduction, which makes them sensitive to inbreeding depression by the formation of diploid males. Thus, different strategies should have been evolved in these species to avoid sib-mating and the deleterious effects of inbreeding depression. Some of these Braconidae parasitoids are solitarily distributed in the environment, which is known to decrease the probability of sib-mating within a population. Other species, such as aphid parasitoids, are more clumped in the environment. As their host lives in groups, when a female lays its eggs in an aphid colony, a synchronous emergence of its offspring may result in local mate competition and high risk of inbreeding.

Aphid parasitoids have probably developed some mechanisms to avoid mating between siblings or competition between brother males.

In this context, our main hypotheses are first that that aphid behaviour after parasitation may influence the parasitoids mating structure (defined here as mechanisms acting before pair formation). Secondly, we hypothesize that aphid parasitoids have developed adapatations to avoid inbreeding and local mate competition (defined here as mechanisms acting during pair formation).

1. Mechanisms of avoidance of inbreeding and Local Mate Competition acting before pair formation.

Aphids are disturbed by parasitoids and predator behaviour and are known to disperse from their initial patch to another. Our hypothesis is that aphid dispersal behaviour due to parasitism behaviour of the female *A.*

matricariae decreases the Local Mate Competition level. To confirm our hypothesis, we observed the behaviour of *M. persicae* aphid colonies disturbed or not by parasitoid females, from few minutes after the attack to the moment of the mummification of parasitized aphids. Our **prediction 1** is that aphids disperse after the attack of the parasitoid female, so that mummified parasitized aphids move out of the location of the attacked colony. This should induce a non-clumped distribution of mummies (see the patch problem section for our definition of a patch). Results are reported in the paper 1.

Protandry (the emergence of males before females) is widespread in insects and could have been selected to avoid mating between close related individuals. Our second hypothesis is that the rhythm of emergence of *A. matricariae* favours a low level of Local Mate Competition. To confirm this, we investigate the emergence rhythms of *A. matricariae* in laboratory conditions with different photoperiods and observe the post-emergence behaviour of males and females. Our **prediction 2** is that the variability in the emergence pattern and post-emergence behaviour between males and females increases the outbreeding probabilities and decreases the level of Local Mate Competition. Results are reported in the paper 2.

2. Mechanisms of avoidance of inbreeding and Local Mate Competition acting at pair formation time

Polyandry is sometimes selected to dilute the effects of mating between siblings (Pusey & Wolf, 1996) and is associated with gregarious parasitoids' mating system (Ridley, 1993). Our hypothesis is that aphid parasitoids should be polyandrous to decrease the effect of sib-mating. Our main **prediction** is that *A. matricariae* females should be polyandrous. However, the alternative hypothesis could be that they could be monoandrous if the inbreeding probability is low enough due to mechanisms acting before pair formation, or because the optimal choice for the female could be to accept to mate with a sib instead of remaining unmated. We evaluated the degree of polyandry of *A. matricariae* females in different situations known to favour polyandry in insects. To test our predictions, we evaluate the number of mates that a female accepts in various conditions know to favour remating in polyandrous speices. Results are reported in the paper 3.

A difference in the mating window can help males and females of the same brood not to mate together (Pusey & Wolf 1996). Our hypothesis is that the sexual maturation time of males and females should decrease the level of Local Mate Competition. Our second hypothesis is that both sexes

should accept to mate with an old partner if they themselves are older, but should be choosier when young. We thus evaluated the mating window of both sexes and test mate choice in relation to age in both sexes. Our **prediction** is that males and females have a delayed sexual maturation time, favouring pre-mating dispersal as virgin from the patch and decreasing the level of Local Mate Competition because females have a higher maturation time. We also predict that virgin females and males should not be very choosy and accept to mate with an old partner.

Direct sib-recognition could be selected to avoid sib-mating as in the gregarious species *B. hebetor* (Ode *et al*. 1995). As aphid parasitoids potentially emerge under Local Mate Competition, we hypothesize that direct recognition and avoidance of brother-sister mating is selected. We thus tested the mate choice of both sexes in relation to the degree of inbreeding. Our **prediction 6** is that *A. matricariae* should have kin-recognition and avoidance.

The different experiments are summarized in the figure 9.

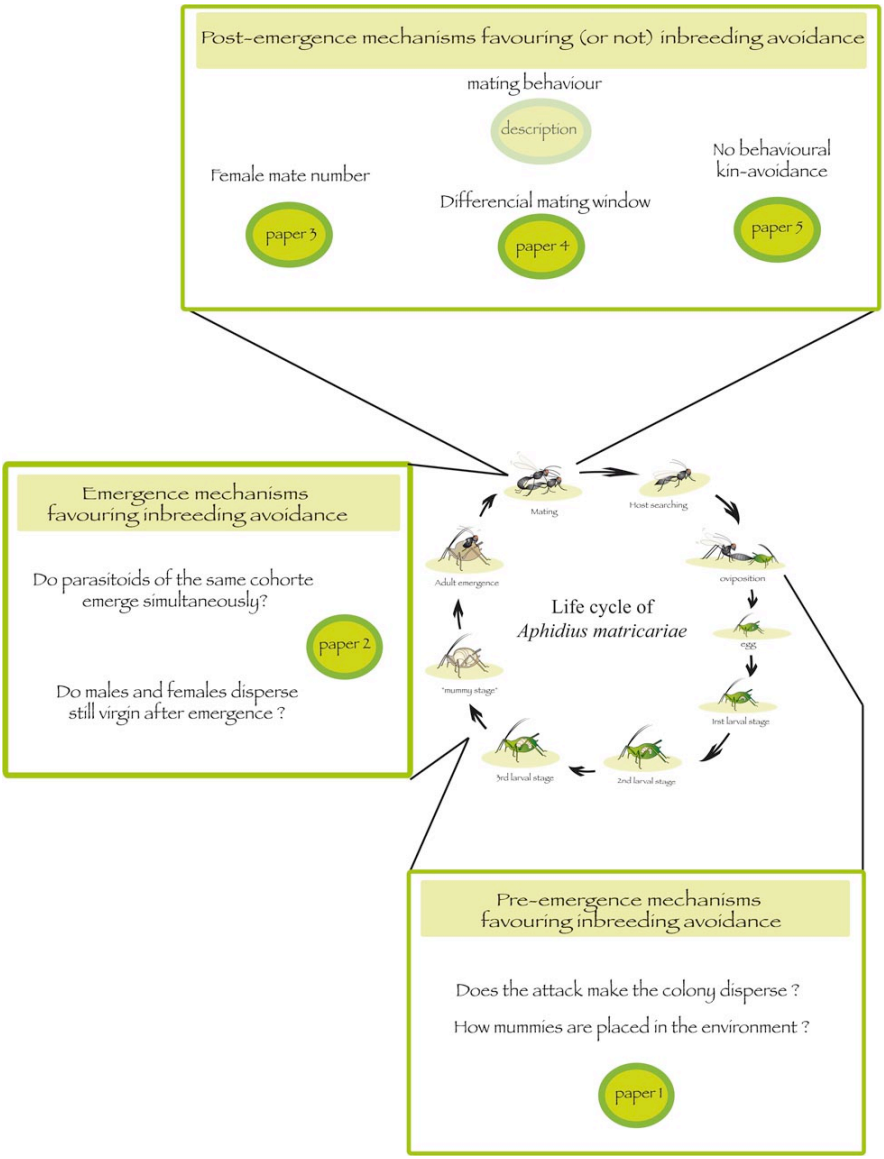

Figure 9. Schematic view of the different experiments of this work.

Chapter II. The organisms used as models

The organisms used in this study form a tri-trophic system composed of:

- The plant: the turnip (*Brassica rapa* var. *rapa*)
- The phytophagous insect: the green peach aphid (*Myzus persicae* Sulzer, Homoptera, Aphididae)
- The parasitoid: *Aphidius matricariae* Haliday (Hymenoptera, Braconidae, Aphidiinae)

1. The parasitoid, Aphidius matricariae *(Hymenoptera, Braconidae)*

Among all aphid parasitoid species of the Aphidiinae sub-family we choose *A. matricariae* as a model for our study for the following reasons: *A. matricariae* is a generalist species (see below) that parasite the peach potato aphid *Myzus persicae*. This is an advatage because we can use an artificial media to rear the aphid speices, which allows a standardized food intake of the host. *A. matricariae* is thus currently and easily reared in our laboratory. The speices is used in biological control programs to control various aphid species (Boivin *et al.* 2012). Moreover, a better unserstanding of its mating strategies should be also interesting in the management of mass rearing programs for biological control purposes. However, contrary to other *Aphidius* species, *A. matricariae* has been poorly studied and most of the data we collected in the literature date from before 1980'. Later, some studies reported the species, but more in relation to its potential use as a biocontrol agent against aphids than in fundamental studies.

A. matricariae (Figure 10) belongs to the Aphidiinae, a highly specialized Hymenoptera sub-family in witch all species only parasitize aphids (Haliday 1834, Hagvar & Hofsvang 1991, Kambhampati *et al.* 2000). A general life cycle of the Aphidiinae is given in figure 11.

Figure 10. Example of some behaviours of *Aphidius matricariae*. (a) mating (b) a female parasitizing a larval stage of the aphid *Myzus persicae*. ©D.Bourdais

Geographic distribution

Aphidius matricariae is probably originated from northern India or Pakistan, but is now reported in a total of 81 countries around the world, from Australia, North America and various parts of Europe. Records also include Mongolia, Peru and Brazil (Stary 1975, Schlinger & Machauer 1963, Yu *et al.* 2005). Field samplings in Montpellier in 1995 reported that this species was the most abundant parasitoid of *D. noxia* in this part of France (De Farias & Hopper 1997). The parasitoid was accidentally introduced into North America one century ago (Schlinger & Mackauer 1963). It has been found some years ago at Marion Island (South Africa) as an invasive species (Lee *et al.* 2007). *A. matricariae* was also accidentally found on the rosy apple aphid *Dysaphis plantaginea* in Belgium since 2004 in an apple Orchard in Belgium (Dumont *et al.* 2005).

Host range

A. matricariae is reported to attack around 40 aphid species belonging to several aphid genera (Schlinger & Mackauer 1963, Kalina & Stary 1976). It can be thus considered a generalist aphid parasitoid. This species is a significant parasite of the green peach aphid *M. persicae* (Scopes 1970, Wyatt 1970, Tremblay 1975, T'Hartv *et al.* 1978, van Tol & van Steenis 1994). It was also reported to efficiently parasite *Aphis gossypii* (Zamani *et al.* 2007), *Capitophorus carduinis* (Van Veen *et al.* 2002), *Aphis fabae* (Tahriri *et al.* 2007) or *Diuraphis noxia* (De Farias & Hopper 1997). It is currently use in biological program (Boivin *et al.* 2012, Bannerman *et al.* 2011)

General biology

A. matricariae is a solitary species from a physiological point of view because even if more than one egg is laid in the host, only one individual will complete its development in the host and emerge as an adult

(Vevai 1942, see figure 11 for the life cycle). The adult female laid eggs in the body of the aphid. The egg is elliptical, white, length varying from 0.05 to 0.07mm and breadth from 0.013 to 0.023mm (Vevai 1942). Aphidius' eggs are hydropic, yolkless and contain few energetic reserves, except some lipoid globules, meaning that they do not represent a high energetic investment for the female (Sabri *et al.,* 2011). It appears that between 3 and 5 larval stages are reported for the species, based on morphological observations (Vevai 1942, Muratori *et al.* 2004). The sharp abdomen ended by the ovipositor in the female whereas it is obtuse in male is the main characteristics used to distinguish adult males from females. Moreover, antennae of males (17 to 19 segments) are longer than those of females (14 to 16 segments) (Giri *et al.* 1982).

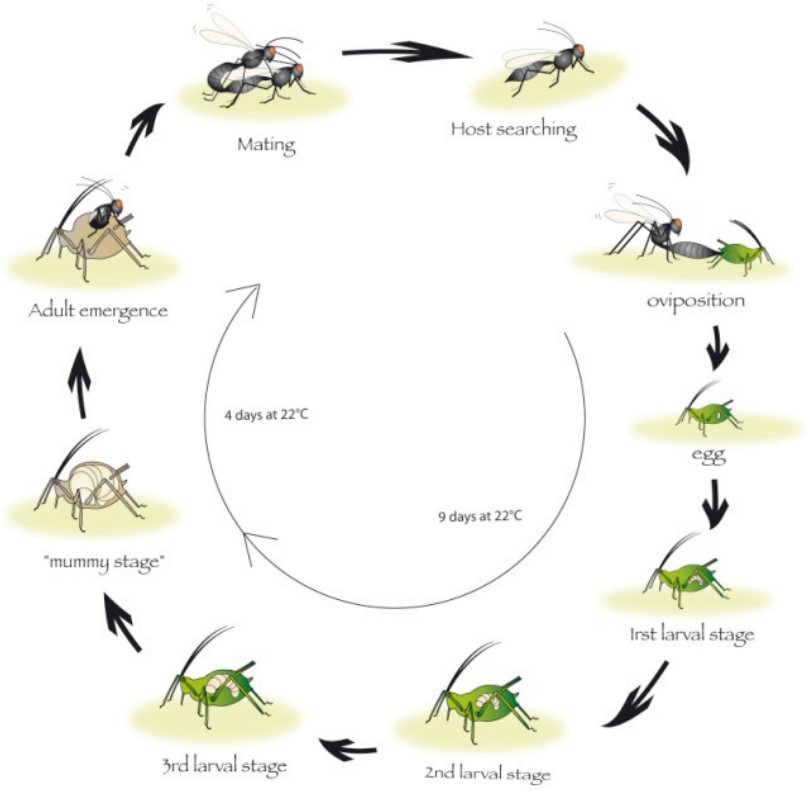

Figure 11. Life cycle of *Aphidius matricariae* parasitizing *Myzus persicae* ©D.Bourdais

As for all insects, the rate of development depends on temperature. A study of Zamani *et al.* (2007) compared developmental rates of *A. matricariae* at different constant temperatures from 5 to 35°C using *A.*

gossipy and *M. persicae* as host. No development was observed at 5°C and 35°C (Table 2). However, when we compared the different developmental times at constant temperature, we observed differences among studies, probably due to the genetic differences in populations or the host aphid species (Table 2).

Table 2. Developmental total duration time of *Aphidius matricariae* at different temperatures and on various host species.

Aphid host	Number of days of development from oviposition to adult at different constant temperatures						Ref. of the study
	5°C	10°C	15°C	20°C	25°C	30°C	
Myzus persicae	-	38.2±3.6	34.8±2.7	18.8±1.4	-	-	Rabasse & Shalaby 1980
	-	54.3±2.4	27.7±2.03	14.8±1.65	11.6±1.09	12.3±1.81	Zamani *et al.* 2007
	-	-	-	11.3±0.02	-	-	This study
Aphis gossypi	-	58±4.46	26.3±3.05	13.7±1.02	11.1±0.86	12.1±1.40	Zamani *et al.* 2007

Under lab conditions, the highest number of offspring a single female can produce varies between 300 and more than 400 individuals depending of authors (Vevai, 1942; Shalaby & Rabasse, 1979; and Giri *et al.,* 1982). Under our lab condition, females lay between 23 and 419 eggs during their lifetime suggesting a high variability among females (n = 23 in our experiment). To date, no study reports if the species is pro-ovogenique or not. *A. matricariae* do not parasitize the first instar nymphs of aphids (Tahriri *et al.,* 2007) but they oviposit in the other nymphal instars and adults. They prefer the third nymphal instar when they have the choice (Talebi *et al.,* 2006; Tahrori *et al.,* 2007). Females show a type II functional response (Tahriri *et al.,* 2007; Zamani *et al.,* 2006). Type II response has been reported in other Aphidiinae species (Mackauer, 1983; Cloutier & Holling, 1984; Liu, 1985; Ives *et al.,* 1999).

The longevity of adults decreases as the temperature increases and males usually live longer than females at lower temperatures (Giri *et al.,* 1982). Adults provided in food and water are supposed to live around 10 days at 20°C (Giri *et al.,* 1982). In a study of the consequences of heat and cold shocks on the species, we estimated the longevity of both sexes in our lab conditions under different temperatures and different food regimes (Jerbi *et al.,* in prep). Results concerning longevity are reported in Table 3. As in the study of Giri *et al.* (1982), we observed that males often live longer than females.

Table 3. Longevity (mean number of days ± s.e.) of males and females of *Aphidius matricariae* at different temperatures and different food regimes (no water and no food, only water, only food, both water and food). Food is a small drop of honey and water a small piece of cotton humidified. 30 individuals of each modality were used (Jerbi M., Bourdais D., *et al.* in prep.).

Food regimen	sex	10°C	15°C	20°C	25°C	30°C
No water	♀	5.8±0.2	2.9±0.3	3.5±0.2	2.5±0.1	1.0±0.0
No food	♂	13.2±0.6	4.9±0.1	4.0±0.2	2.7±0.1	1.1±0.6
Water	♀	13.3±1.9	3.7±0.4	3.9±0.1	4.33±0.1	1.5±0.1
No food	♂	10.2±0.8	11.7±0.3	7.6±0.4	5.3±0.2	3.6±0.3
No water	♀	29.7±3.1	11.8±1.2	4.2±0.1	2.8±0.1	1.4±0.1
Food	♂	53.2±3.4	4.9±0.2	5.8±0.3	2.9±0.1	3.1±0.1
Water	♀	29.0±1.9	15.1±1.4	12.9±1.3	10.4±0.7	5.5±0.6
Food	♂	26.1±3.1	16.4±1.2	13.1±0.8	9.7±0.5	7.8±0.5

Courtship and mating behaviour

Despite the poor precise data that exists, courtship and mating behaviour are reported to be similar to other *Aphidius* species (Giri *et al.,* 1982; Fox *et al.,* 1967; Bourdais *et al*., 2012). To describe mating behaviour, it is useful to split it in separate events with terms as unambiguous as possible. The records produced should accurately reflect the dynamics of behaviour. Here we present results of our observations of the typical sequence of postures of both sexes during courtship and mating in *A. matricariae* (Figure 12).

Authors that had previously worked on mating behaviour of *A. matricariae* found that a low proportion of males are inactive (Giri *et al.,* 1982, 7%, n = 200). The sexually active males exhibited courtship behaviour by fanning of wings within 2 sec. to a few minutes after exposure to females. The male actively pursued the female and usually grasper her by the thorax with its prothoracic legs. During the mating activity, the antennae of the male were used to palpate the antenna of the female. According to the study of Giri *et al.* (1982), the **duration of the copulation** was from 45sec. to 3min. but was usually completed after 50 to 60 sec., but no indication of the number and the age of observed individuals was reported.

The **mating window** of the species seems more controversial. The study of Giri *et al*. (1982) found that the higher proportion of mating appears in young individuals aged from less than one hour, whereas Shalaby & Rabasse (1979) and Vevai (1942) evaluated at 2h the sexual maturation time, which means that no mating can occur before. Mating window of *A. matricariae* was studied in our lab conditions and results are a part of the

paper intituled “Mature males still sexy in the aphid parasitoid *Aphidius matricariae*”.

All authors usually agree with the fact that males are **polygynous** and females are **monoandrous** (Giri *et al.* 1982, Verai 1942) even if few observations were done to prove this statement. However, because the number of partners that a female can accept is important to understand mating system and mating strategies of a species, we decided to investigate more precisely this question (paper intituled “Shift in mating strategy with oviposition in *Aphidus matricariae* females”).

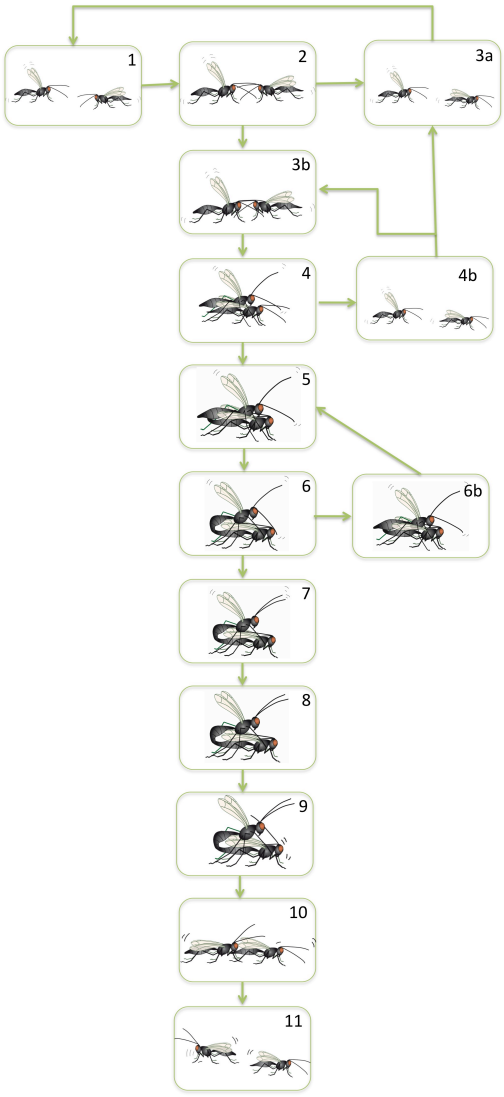

Figure 12. Mating behaviour in *A. matricariae*. The diagram shows interactions between males and females in typical courtship. Males and females used to describe this flow diagram were 24h old and virgin, 30 matings were observed. Behaviour abbreviations are defined in Table 4 and adapted from the mating sequence described in *A. rhopalosiphi* (Bourdais *et al.* 2012).

Table 4. Description of the behavioural components of *A. matricariae* males and females during the courtship and mating process described in figure 12. The number of each line corresponds to the number of the schematic representation in figure 12.

	Behaviour of the male	Behaviour of the female
1	Walking with its wings up (wing fanning) in alternation with walking period with its wings down	Moving forward, usually alternatively touching the antenna with the substratum
2	Antennal contact with the female	No change in behaviour
3a	No change in behaviour	The female escapes from the male contact
3b	The male continues to have antennal contact with the female	The female stays
4	The male mounts the female and makes antennal movements in alternation when it is on the back of the female	No change in behaviour, the female does not move
4b	No change in behaviour	The female moves and tries to escape
5	The male continue to makes antennal movements alternating both antenna	The female changes its position and raises its abdomen
6	The male lowers its abdomen until the genitalia contact	The female accepts the contact
7	Contact between the genitalia	Contact between the genitalia
8	The male flicks its antennae in parallel and keeps them in contact with those of the female	No change in behaviour
9	The male is redressed on the back of the female but does not lose antennal nor genitalia contact	No change in behaviour
10	No change in behaviour	The female moves and escapes
11	The males escapes	

Rearing method: how to obtain size standardized adults ?

Aphidius matricariae was originated from Italy and is commercialized by the company Viridaxis.

The species is reared on *Myzus persicae* in climatic room (22°C) with a summer like photoperiod (16 hours of light and 8 of night). Because adult size can have a great impact on behavioural decisions of both sexes in insects (Bonduriansky, 2001; Joyce *et al.* 2009), adult parasitoids are allowed to parasitize aphids of a given size to standardise the size of offspring. This rearing method allows us to obtain size-standardized individuals (see annexe 1).

2. The aphid Myzus persicae *(Homoptera, Aphididae)*

General biology

The green peach aphid, *Myzus persicae* (Sulzer) (figure 13), is found throughout the world and is viewed as a pest principally due to its ability to transmit plant viruses. In addition to attacking plants in the field, green peach aphid readily infests vegetables and ornamental plants grown in greenhouses.

Figure 13. Various stages of the peach potato aphid *Myzus persicae*. a : winged adult, b : larvae ©D.Bourdais

The **life cycle** varies considerably, depending on the presence of cold winters. Van Emden *et al.* (1969) provides a good review of the life cycle (Figure 14). Development can be rapid, often 10 to 12 days are needed for a complete generation and over 20 annual generations may occur under temperate climates of North Europe.

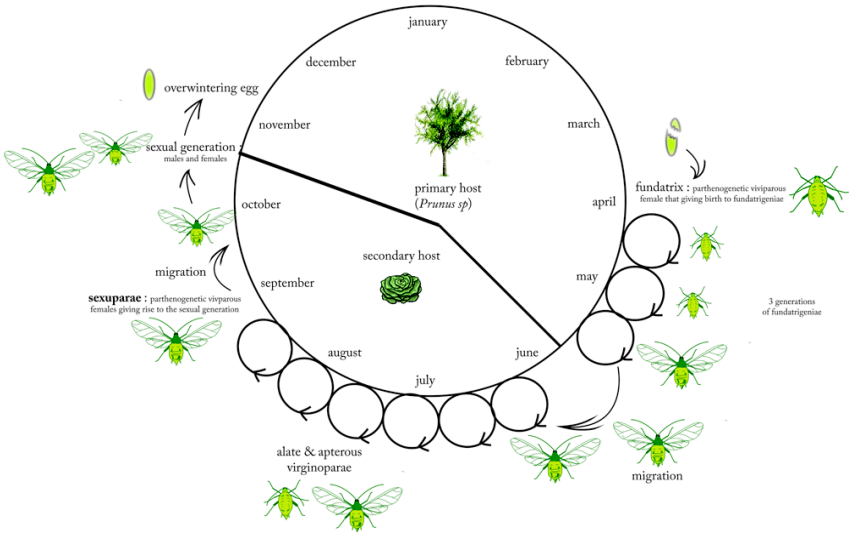

Figure 14. Life cycle of *Myzus persicae* in temperate zones. ©D.Bourdais

Green peach aphids feed on hundreds of host plants in over 40 plant families. In temperate latitudes the primary host (or overwintering hosts) are trees of the genus *Prunus*, particularly peach and peach hybrids, but also apricot and plum. During the summer months, the aphids abandon their woody hosts for secondary or herbaceous hosts, including vegetable crops in the families Solanaceae, Chenopodiaceae, Compositae, Cruciferae and Cucurbitaceae. Vegetables that are reported to support green peach aphid include beets, broccoli, Brussels sprouts, cabbage, turnip, pepper, potato, radish, turnip, etc.

Green peach aphids can attain high densities on young plant tissue, causing water stress, wilting, and reduced growth rate of the plant. Prolonged aphid infestation can cause appreciable reduction in yield of root crops and foliage crops. The major damage caused by green peach aphid is through transmission of plant viruses. Indeed, this aphid is considered by many to be the most important vector of plant viruses throughout the world (Blackman & Eastop, 2000). Nymphs and adults are equally capable of virus transmission (Namba & Sylvester, 1981), but adults, as they are more mobile, probably have greater opportunity for transmission. Kennedy *et al.* (1962) listed over 100 viruses transmitted by this species. Some of the particularly damaging diseases include potato leafroll virus and potato virus Y to Solanaceae, beet western yellows and beet yellows viruses to Chenopodiaceae, cauliflower mosaic and turnip mosaic viruses to Cruciferae.

Rearing method

Myzus persicae is reared in our lab under the same conditions as its parasitoid. The strain is maintained on turnip plant but for our experiments we used an artificial media provided by Viridaxis S.A. This media creates a standardized supply in food nutriments and decreases effect of food intake on the parasitized aphid.

3. The host plant, Brassica rapa var. rapa

Cultivated in Europe for over 4000 years, probably native to central and southern Europe, it is now spread throughout world, including most parts of the tropics.

The use of *Brassica rapa* for this study as a host plant was yield by two points:

(1) Its richness in constitutive direct defences (glucosinolates) and in induced defences (CVH). When attacked by herbivorous arthropods, turnips are known to release various types of volatiles that attract natural enemies of these herbivores. These herbivores-induced plant volatiles are highly detectable and reliable cues for foraging natural enemies as it was yet shown for various parasitoid species (Neveu *et al.,* 2002).

(2) It is an adequate good host plant for our aphid strain that develops well on it. We tried to rear our strain of aphid on various plants: potato var. Nicolas and Désirée, peper, *Vicia faba*, turnip but *M. persicae* thrives preferably on turnip plants.

Laboratory culture

Turnips are classically reared in a greenhouse and were obtained from seeds.

Chapter III. Inbreeding avoidance mechanisms acting before pair formation

1. General introduction

Understanding the mating structure of a species and especially how it behaves towards inbreeding does not only required to understand the behavioural mechanisms that are selected during or after mating, but also to understand the spatio-temporal structure of both adult and larval populations.

In aphid parasitoids, the succeptibility to inbreeding depression is due to their sex-determination rules (Heimpel & De Boer, 2008). Thus, some mechanisms of inbreeding avoidance should decrease the sib-mating opportunities before or at the moment of adult emergence. These mechanisms will be influenced by:

- The exploitation strategies of aphid colonies by females
- The behaviour of parasitized aphids
- The synchronization of sex at emergence
- The post emergence behaviour of both sexes

Aphid populations are not homogeneously distributed in the environment and female parasitoids exploit a succession of different colonies (Le Ralec *et al.,* 2010). A single female often parasitizes aphid colonies. They then undergo a phase of population growth based on parthenogenetic reproduction followed after several generations by the collapse of the colony due to predators, parasitoids or weather conditions (Hance, 1995; Le Ralec *et al.,* 2010). It is at this stage that alate aphids can disperse and create a new colony. According to the theories of optimal foraging developed in behavioural ecology (Optimal Foraging Theory; Charnov, 1976), parasitoid females should allocate adequately the time for laying their eggs and the time to find host patches. The female is expected to adjust its exploitation of the aphid patch (number of aphids to parasitize, sex ratio…) using different factors such as the number of aphids per patch, the travel time between two colonies or the quality of the colony (number of parasitized aphids) (Wajnberg *et al.,* 2008). In quasi-gregarious species, the female should optimize the sex-ratio of the brood according to the level of competition that its sons will encounter at emergence, following the Local Mate Competition rules described in the introduction part.

Once the colony parasitized by one or several females, the future localization of adult parasitoid emergences may be influenced by the **behaviour of parasitized aphids.** The examples of manipulation of the host by a parasite are numerous (see Moore, 2002; Thomas *et al.,* 2005), including for parasitoids (Brodeur & Boivin, 2004). The behavioural changes observed following parasitism mostly benefit the parasite (increase

of its chances of survival - Brodeur & McNeil, 1989; Biron *et al.,* 2005) but could also be beneficial to the host by increasing its "inclusive fitness" (Smith Trail, 1980). Aphids are known to have altered behaviours due to the immature parasitoid presence in their body (Behrendt, 1971; McAllister *et al.,* 1990; Müller *et al*., 1997; etc…). However, observations of aphid-modified behaviour had never been exploited in relation to the reproductive strategies of the parasitoid.

In this work, we thus tested the behaviour of aphids parasitized by *A. matricariae* and interpret the results in relation with the mating system of the parasitoid. Unlike gregarious parasitoids, adult parasitoids that emerge from mummies scattered in space (resulting from the dispersal of parasitized aphids), have little chance to emerge in the same place and same time of a potential partner. However, if the mummies are aggregated in the aphid colony, the pattern of emergence could be closer to a gregarious pattern with on-patch mating and inbreeding. In practice, there are two reasons why aphid may disperse after parasition. First, when a predator or a parasitoid visits an aphid patch, aphids emit an alarm pheromone which usualy results in dispersal. In thaht case, parasitized aphids probably react as non-parasitized ones, at least at the very beginning of parazitation. Secondly, parasitized aphid may show an altered behaviour and disperses from its patch whether it is due to kin selection or parasitoids manipulation. In consequence, we expected that mummies would not be clumped at the moment of the parasitoid emergence. Results of this study are reported in the paper 1.

If spatial distribution at emergence is a major issue for inbreeding avoidance, timing of emergence of brothers and sisters of the same cohort could also limit the probability of meeting and mating on the emergence patch. For instance, protandry (i.e. defined as the emergence of males before females) is a commonly found in many species including parasitoids (Saunders, 2002; Pompanon *et al.,* 1995). As it was already shown in *A. ervi* (He *et al.,* 2004), we expect that males in *A. matricariae* have a shorter development time than females. Moreover, we wished to evaluate how emergence rhythms could favour or not mating between relatives by the simultaneous emergence of brothers and sisters. Indeed, this important aspect in mating systems had never been explored in aphid parasitoids. Results of this study are reported in the paper 2.

Finally, **post-emergence behaviour of both sexes** should also be taken into account when looking at population structure in order to understand the structure of the mating and the relation with sibmating avoidance. The patterns of dispersion from the patch may also have an influence on the probability of mating between partners. Staying on the emergence patch and waiting for the emergence of a partner will increase the probability of inbreeding, whereas quickly disperse from the patch will increase the probability of remaining virgin (Martel & Boivin, 2004). In *A.*

matricariae, as in many aphid parasitoids, nothing is known about the dispersal behaviour and post-emergence of both sexes. We evaluate this behaviour in the particular conditions of a big synchronized mummy patch and results are included in the paper 2.

To summarize, to understand the mating strategies of *A. matricariae*, we first focus on the structure of the population at the moment of the emergence of individuals. Our basic assumption is that parasitized aphids disperse from the colony. Together with protandry and a rapid dispersal of males shortly after emergence, the encounter rate between partners on the emergence patch should favour mating outside the patch, reducing in the same time the risk of inbreeding.

Thus, our prediction is that a combination of scatter distribution, protandry, non-synchroneous emergence and rapid dispersal from the natal patch were selected in *A. matricariae* to decrease the risk of sib encounters after their emergence.

2. How mummies of A. matricariae *are distributed in the environment after an attack of a* M. persicae *colony by parasitoid females?*

Paper 1

Aphid dispersal after a parasitoid attack favours a solitary emergence of the female parasitoid offspring

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Submitted to Environmental Entomology

Abstract

Several mechanisms change the spatial distribution of an aphid colony after parasitisation. Indeed, when a parasitoid female lays her eggs into aphids, they react in various ways (dropping from the host plant, kicking, walking out of the colony…) because of the emission of an alarm pheromone. However, as aphid dispersal modifies the spatial localisation of parasitoids emergence, it could affect the mating strategies of the parasitoid. We investigated the spatial localisation of mummies of the parasitoid *Aphidius matricariae* following an attack of patches of the aphid host *Myzus persicae* and discussed the results in regard to the mating strategies of the parasitoid. It can be expected that if aphids do not disperse after a parasitoid attack of the colony, mummified aphids will stay clumped. Emergence pattern of the next parasitoid generation will correspond to a quasi-gregarious situation with a high level of local mate competition between sibs. In practice, we observed that aphids disperse from the initial colony resulting in an isolation of mummies on a leaf most of the time. It is only when high aphid density patches are used that formation of small groups ($n<10$) of mummies is observed. Most of the time *A. matricariae* will emerge at a distance of a potential mating partner stressing the importance of searching for a mate out of the natal patch. Moreover in post-emergence behaviour observations, males and females tend to disperse rapidly from the emergence patch, which also increases the probability of out-patch mating.

Key words.

Spatial distribution, *Aphidius matricariae*, aphid dispersal, inbreeding avoidance

Introduction

Spatial distribution of organisms influence many life history traits and determine the way the behavioural strategies have evolved (Anfdrawartha & Birch, 1954). It notably conditions the encounter probability between males and females in relation with inbreeding avoidance. This is particularly the case in gregarious insect for which dispersal at the onset of adulthood is a way to avoid sib mating. In koinobionts parasitoids, the place of adult emergence depends on the host behaviour. When host are clumped, solitary parasitoids are considered as quasi-gregarious species. However, the host behaviour after the parasitoid attack can change (e.g. host dispersal) and induce important consequences on the spatial population structure of its parasitoid (Mackauer & Völkl, 1993; Brodeur & Rosenheim, 2000), which are directly linked with mating strategies (Le Ralec *et al*., 2010; Fauvergue *et al*., 1999). Mating systems of parasitoids are traditionally concerned with the issue of whether mating is restricted and local or random and panmitic and influence by the genetic relatedness between mates (Godfray & Cook, 1997). Females of solitary species lay a single egg in each host and competition for access to mates may occur throughout the whole population. Conversely, in solitary parasitoids that develop quasi-gregariously and gregarious parasitoids, brothers compete for a limited number of females to mate, through a process called Local Mate Competition (Hamilton, 1967; Franck, 1990; Antolin, 1999). Fully local mating may frequently be incorrect in many species because males are potentially capable of dispersing after emergence, and females can also disperse still virgin (Nunney & Luck, 1988; Martel & Boivin, 2004). Mating structures intermediate to the extremes of panmixis and fully local mating may be common. Spatial distributions of emergence of adult parasitoids are important aspects of mating systems, but this aspect is still neglected for most of parasitoid species.

Aphid parasitoids (Hymenoptera: Braconidae) are traditionally considered as quasi-gregarious species with partial local mating (Godfray, 1994; Schwörer & Völkl, 2001; Maukauer & Volkl, 2004; Wajnberg, 2006). Aphids are patchily distributed in colonies in the environment and parasitoid females will exploit host patches, laying male and female eggs in different aphids of the same colony (Müller *et al.,* 1999; Brodeur & Rosenheim, 2000; Le Ralec *et al*., 2010). Offspring are thus expected to emerge simultaneously (Beck, 1991; He *et al.,* 2004; Doyon & Boivin, 2005) and brothers compete for access to their sister to mate (Local Mate Competition; Hamilton, 1967). However, the biology of their host has to be taken in account to really understand if host behaviour after parasitism may alter parasitoid mating system. Aphids often directly react to the host exploitation behaviour of the parasitoid female (Vandermoten *et al.,* 2012). Disturbed aphids secret

droplets of fluid that contain an alarm pheromone composed of E-β-Farnesene that act as a signal favouring moving away from it and often drop from the host plant (Pickett *et al.,* 1992; Vandermoten *et al*., 2012). After parasitism, the aphid host is not killed immediately but continues to feed, grow, moult and potentially disperse. The larva develops as a solitary endoparasitoid, feeding selectively on its host tissues and organs (Polaszek, 1986; Sabri *et al.,* 2011). The last larval instar kills the aphid and pupates either inside or in a separate cocoon, below the mummified host (Stary, 1970; Sabri *et al.,* 2011). Because of these particular life history traits, aphids can express defensive behaviours not only during the attack of the colony but also after parasitism. The parasitoid could modify the behaviour of its host for its own fitness (Fritz, 1982; Brodeur & Boivin, 2004) through the decrease of hyperparasitism probabilities (Sullivan, 1988; Brodeur & Mc Neil, 1992; Mackauer & Völkl, 1993) or to find better overwintering sites (Brodeur & McNeil, 1989; 1990). It is thus quite well known that aphids disperse after the parasitoid attack but to date we found no reference about post-attack behaviour of aphids in relation with parasitoids emergence structure and relation to partial local mating.

Aphidius matricariae (Hymenoptera, Braconidae) belongs to the Aphidiinae (Hagvar & Hofsvang, 1991). It is reported to attack around 40 aphid species belonging to several aphid genera (Schlinger & Mackauer, 1963; Kalina & Stary, 1976). It can be thus considered as a generalist aphid parasitoid and is commercially produced to control various aphid species including the peach potato aphid *Myzus persicae* (Boivin *et al*., 2012). *M. persicae* is a worldwide aphid pest, attacking more than 40 plant families and responsible of huge damages in cultivar crops such as beets, cabbage, turnip, pepper, potato, radish, mostly due to virus transmission. *M. persicae* is known to increase its mobility in response of fungal infection (Roditakis *et al.,* 2007). It is also known to react to the alarm pheronome (E-β-Farnesene) by dispersing from the plant when attacked by predators (Belliure *et al.,* 2011) but no data exist about its behaviour when parasitized.

In this paper, the dispersal behaviour of *M. persicae* after an attack of the colony by *A. matricariae* parasitoid females and the distribution of the mummies induced by this behaviour has been studied in relation with mating strategies of the parasitoid. We conducted 2 major experiments to understand the dispersal behaviour of the aphid and its consequences on the parasitoid structure. First, we observed the dispersal of aphids after an attack of the parasitoid females during a short time interval (96 h) in order to understand if the dispersal is rapid after the attack or takes more time. In the second experiment, we tested at a larger time scale (10 days) the influence of the size the initial aphid colonies on the mummies distribution on the different turnips that compose our experimental area. This was conducted to understand how the mummies are distributed in the environment after an

attack of a single aphid colony but also how males and females are distributed. According to the literature, we predicted that because of the dispersion of aphids from the initial colony, males and female parasitoids emerge alone, meaning that they behave more as solitary species than true quasi-gregarious ones. The consequences of this emergence pattern will be a lower Local Mate Competition than in other quasi-gregarious species (Godfray, 1994; Hardy, 1994).

Material & method

Insect material.

In the laboratory, *Aphidius matricariae* was reared on the peach potato aphid *M. persicae*, maintained on turnip plants. We use young turnip plants obtain from seeds of the variety "blanc à collet violet". All turnips used for the experiments had 6 leaves well developed. Insect colonies were established by using material provided by Viridaxis S.A. (Belgium) that use an Italian strain for commercial mass rearing. Aphid and parasitoid cultures were maintained under 20 ± 2C°, 70 ± 5% r.h. and L16:D8. To standardize parasitoids in size and thus avoid any effect of size on patch exploitation strategies of the females, newly emerged mated *A. matricariae* females were isolated and allowed to parasite a maximum of 100 2nd stage aphids (aphids aged between 2 and 4 days) placed in a Petri dish (Ø=9cm) during 4h. To obtain naïve individuals for bioassays in cages, all parasitoid pupae (i.e. mummies) from every female were kept individually in 1.5ml microcentrifuge tubes until emergence. The mummies were checked twice daily and we used only males and females that emerged in the morning, between 7 p.m. and 1 a.m.

Experiment 1. Do aphids rapidly disperse after a parasitoid attack?

To study the dispersal of aphids after a parasitoid attack we used lab cages (50 x 50 x 70 cm, n = 10 per treatment, figure 1). In each cage, 9 turnip plants were placed and a colony of 50 aphids aged between 2 and 4 days was artificially created on a leaf of one of the turnip (see figure 1). One hour after the aphid colony formation, 5 parasitoid females (FEM), 5 parasitoid males (MAL) or nothing (CONTROL) was added in the cage. Parasitoids were taken off the cage 4 hours after their introduction. We then observed the number of aphids per turnip plant and number of aphids remaining on the initial leaf (i.e. the number of non dispersing aphids) at various time intervals after the attack (6h., 8h., 10h., 24h., 32h., 48h., 56h., 72h., 80h. and 96 h.). We predict that disturbed aphids will rapidly disperse from their initial patch (= turnip leaf) to adjacent turnip plants when attacked by the parasitoid females while the aphids should remain on the initial leaf in control or in presence of males.

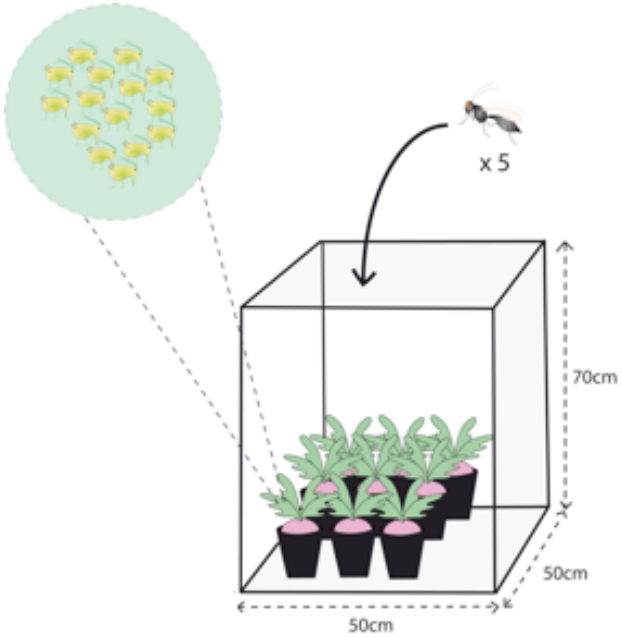

Figure 1. Experimental design in the laboratory cage. The initial aphid colony was placed on the leaf n°1 of the left corner turnip plant and parasitoids were added in the cage when needed.

Experiment 2. Influence of the aphid colony size on the spatial localisation of mummies

In this experiment, we determine if the size of the initial colony of aphids has an influence on various aspects of aphid dispersal and the future localisation of mummified aphids. We used the same lab cages (50 x 50 x 70 cm, n = 12 per modality) as previously. In each cage, 9 turnip plants were placed and a colony of 50, 100 or 300 aphids aged between 2 and 4 days was created on a leaf of one of the turnip (figure 1). One hour after 5 parasitoid females were added in the cage and taken off the cage 4 hours after. 10 days after, we observed the position of mummies per turnip plant and for each leaf. The following characteristics had been recorded for each mummy: the turnip plant number, the turnip leaf number, if the mummy were in a patch of mummies or not. Each mummy was then isolated and the sex of the emerged parasitoid recorded. Because we wanted to observe the mummies positions after an attack of females, we did not make control without females. As predicted in the first experiment, aphids should disperse from their initial turnip leaf and parasitized aphids should mummify out of this leaf. Here, we also predict that the number of aphids initially present on the turnip leaf has a positive effect on the probability to find patches of mummies in the different turnips.

Statistical analyses

In the first experiment, the evolution of the proportion of aphids that dispersed from the initial colony was calculated using a non-linear regression model (exponential decay equation model, GraphPad Prism). The values of the number of dispersal aphids as a function of time fits to a curve following the formula: $Y=(Y_0\text{-Plateau})*\exp(-K*X)+\text{Plateau}$ (GraphPad Prism). In this formula, "Y_0" is the number of aphids in the colony at time zero (fixed at 50), "Plateau" is the number of aphids at infinite times and "K" is the slope of the decreasing phase expressed in $hour^{-1}$.

In the second experiment, as we have only 3 different densities of aphids, we used ANOVAs followed by Tukey post hoc tests instead of regression to analyse the total number of mummies observed per cage, the mean percentage of mummies observed out of the initial turnip leaf and the mean number of patches of mummies observed per cage. As we did not want to see an evolution of the number of individuals under Local Mate Competition in function of the size of the aphid colony, we also analysed data about the number of males and females under LMC or not using one-way ANOVAs followed by Tukey post hoc tests.

All tests wee applied under two-tailed hypotheses and the significance level p was set at 0.05. All analyses were performed using GraphPad Prism version 5.1 for Mac OS (GraphPad software, Dan Diego, CA, USA, www. Graphpad.com).

Results

Experiment 1. Do aphids rapidly disperse after a parasitoid attack?

The introduction of 5 parasitoid females in the cage induces rapid aphid dispersal behaviour (table 1, figure 2) as around a half of the aphids of the colony change of turnip plant only 6 hours after the attack of the female parasitoid which is not observed in male or control conditions (figure 2). The K values analyses (using the K and 95%CI values) confirm that the rate of aphid dispersal from the initial colony is higher when the females were introduced in the cage than when the males or nothing was introduced (table 1). If we observed aphids at a more local scale, 96 hours after the attack by females, only less than 8% of individuals are still present in the initial colony compared to 56% in control (Plateau values, table 1). The presence of males induced aphid dispersal significantly lower than females but higher than in control (table 1, plateau and their 95%CI values).

Table1. Values of the different parameters of the non-linear regression used to analyse the dispersal of aphids after the introduction of 5 parasitoid females (FEM), 5 parasitoid males (MAL) or nothing (CONTROL).

	Modality		
	FEM	MAL	CONTROL
Aphid presence in the initial colony			
Best-fit values			
Y_0	= 50	= 50	= 50
Plateau	3.57	16.44	27.44
K	0.21	0.15	0.08
95% Confidence Intervals			
Plateau	2.24 to 4.91	11.40 to 21.47	25.08 to 29.80
K	0.17 to 0.24	0.05 to 0.26	0.05 to 0.11
Goodness of Fit			
Degrees of Freedom	119	64	97
R^2	0.84	0.29	0.48
Absolute Sum of Squares	4045	15670	5665
Aphid presence on the plant containing the initial aphid colony			
Best fit value			
Y_0	=50	=50	=50
Plateau	19.52	43	47.81
K	0.15	0.20	0.18
95% Confidence Intervals			
Plateau	17.39 to 21.66	41.29 to 44.71	46.95 to 48.66
K	0.10 to 0.19	0 to 0.47	0 to 0.58
Goodness of fit			
Degrees of freedom	119	64	97
R^2	0.50	0.12	0.04
Absolute sum of Squares	9734	1919	1085

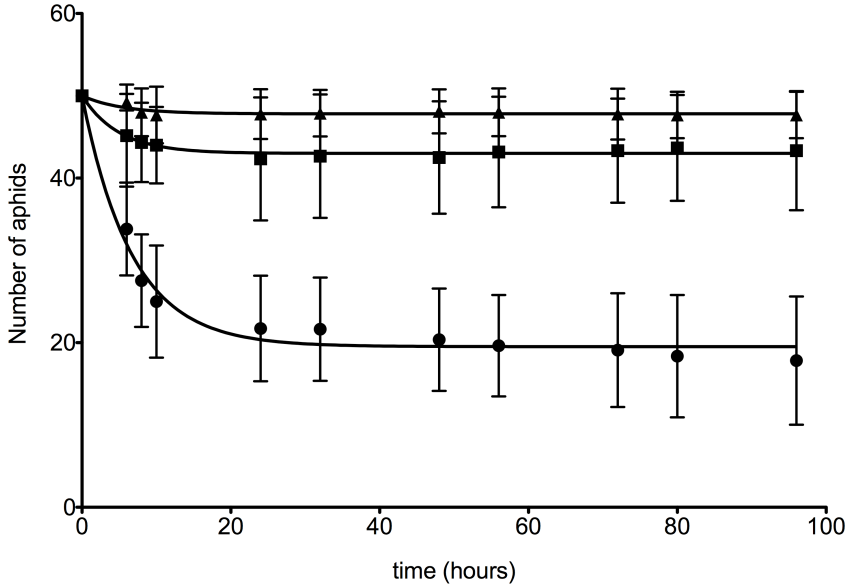

Figure 2. Changes with time (hours) in the mean numbers of aphids founded on the initial plant where the colony was created. Results are given in mean ± s.e. Triangle line represents the control experiment (no parasitoids introduced in cages), Square lines the experiments with male introduction and point lines the experiments with parasitoid females introduction. Parameters of the non-linear regression curves are given in Table 1.

Experiment 2. Influence of the aphid colony size on the spatial localisation of mummies

We observed that bigger is the initial colony, higher is the number of mummies formed (one way ANOVA, p = 0.003, F = 6.91, df = 2, residuals = 32, figure 3a). The sex ratio is constant between cages with a mean proportion of males of 44.97±1.84% per cage (one-way ANOVA, p = 0.67, F = 0.39, df = 2, residuals = 32). The effect of the attack by females *A. matricariae* was the same in terms of dispersal of aphids from their natal colony independently of the size of the colony because no effect of the initial size of the aphid colony was observed on the percentage of mummies founded out of the initial turnip leaf (one way ANOVA, p = 0.55, F = 0.59, df = 2, residuals = 32, figure 3b). A "patch of mummies" is defined in our study as the presence of more than 1 mummy on the same leaf of the turnip. The size of the initial aphid colony has an influence on the number of patches of mummies formed in cages (table 2). Bigger is the initial aphid colony and higher is the number of patches of mummies observed (one way ANOVA, p = 0.001, F = 8.17, df = 2, residuals = 32, figure 3c). However, the number of mummy per patch is constant (table 2). If we take in account

the relation between the mummies patch formation and the mating strategies, we observed that a similar proportion of patches where individuals can emerge under LMC exist independently of the size of the aphid colony (table 2). This mean that around 50% of individuals can emerge under LMC and potentially mate on patch and 50% of both sexes emerge alone and have to find a mate out of their local natal patch.

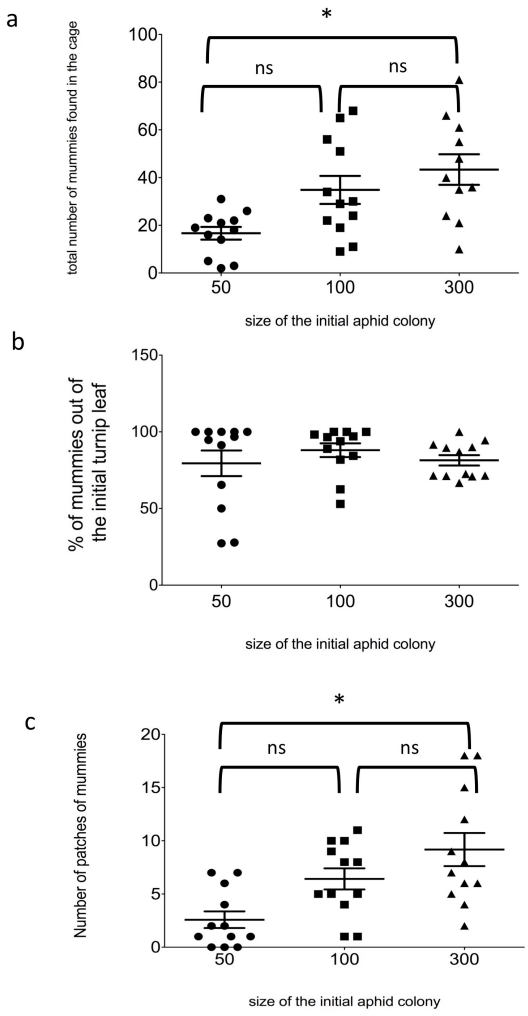

Figure 3. Consequences of the aphid dispersal after the *A. matricariae* female attack on various parameters in function of the size of the initial aphid colony (50, 100 and 300 *Myzus persicae* aged from 2 to 4 days). (a) mean number (± s.e.) of mummies produced, (b) the percentage (± 95%CI) of mummies observed out of the turnip leaf containing the initial aphid colony and (c) the mean number of patches of mummies observed (± s.e.)

Table 2. Localisation of mummies and relation to mating strategies after parasitism of a colony by 5 *A. matricaraie* females. N=12 cages for each density of aphids. A "patch of mummies" is defined as the presence of at least two mummies on the same turnip leaf. Tukey tests between treatments different letters corresponds to a difference at p=0.05

	Aphid colony size			Statistics
	50	100	300	one-way ANOVA
Number (mean±s.e.) of patches of mummies per cage	2.58+0.78a	6.41+0.98ab	9.16+1.55bc	**p=0.001** F=8.17
Size (mean±s.e.) of the patch of mummies	5.92+0.90a	3.59+0.98a	5.48+1.55a	p=0,47 F=0.77
% (mean±s.e.) of patches under LMC	66.11+10.83a	47.58+9.25a	54.83+7.07a	p=0,37 F=1.01
% (mean±s.e.) male emerging alone	60.93+9.15a	48.57+8.88a	43.77+6.61a	p=0.33 F=1.14
% (mean±s.e.) female emerging alone	61.99+8.16a	58.63+8.17a	44.47+6.37a	p=0.24 F=1.49
	2 way ANOVA	Gender	p=0.54, df=1, f=0, ddl=36	
		Colony size	p=0.10, df=2, f=2, ddl=37	
		Interraction	p=0.80, df=2, f=0,22	

Discussion

Myzus persicae disperses from the colony after an attack by *Aphidius matricariae* females. Similar results have been observed when *Myzus persic*ae is disturbed by predators (Belliure *et al.,* 2011) or by fungal infection (Roditakis *et al.,* 2007). When *Aphidius matricariae* female exploits a colony of *Myzus persicae*, the immediate consequence is the rapid (few hours) dispersion of aphids from the colony (experiment 1). One of the consequences of such aphid behaviour is that parasitoid offspring resulting of one exploited colony will not emerge clumped in the environment (experiment 2) as supposed in the literature (Schwörer & Völkl, 2001; Maukauer & Volkl, 2004; Wajnberg, 2006).

When a predator or a parasitoid disturbs an aphid colony, aphids react and often disperse. Dispersal of parasitized individuals could be beneficial for the aphid colony to avoid the future emergence of parasitoids in the colony (Smith Trail, 1980; McAllister & Roitberg, 1987). However, non-parasitized aphids also disperse, probably to avoid the present but maybe also future parasitoid attack. The dispersal behaviour of parasitized and unparasitized aphids can be costly (LeRalec *et al.,* 2010) because of the risks of not to find a new host plant, or desiccation and predation when walking on the ground (Nelson, 2007). Dispersal is probably triggered by the emission of alarm pheromones due to the presence of the parasitoid female

in the colony (Pickett *et al*., 1992; Dixon, 1998), which could explain why parasitized and unparasitized aphids will disperse. Males alone did not induce the same behaviour. The escape behaviour of *Myzus persicae* under an *Aphidius matricariae* females attack the colony should be analysed more deeply. Indeed, here we only used standardized colonies of aphids with individuals at the same stage (between 2 and 4 days old). Some evidences of age dependent sensitivity to the emission and the reaction to the alarm pheromone has been observed (Byers, 2005; Mondor *et al.,* 2000; Schwartzberg *et al*., 2008) and mixed colonies should thus be tested to better fit with real field conditions.

Dispersal behaviour of aphids constrains the mating system of its parasitoids, a point never explored to our knowledge. Usually, one or few females explore a colony of aphids (Schwörer & Völkl, 2001; Nyabuga *et al.,* 2011) resulting in the presence of sibs at emergence of the next generation. Thus, if individuals of the same brood emerge together in space and time, competition for mates on the natal patch will occur and brothers will mate with their sisters (Local Mate Competition; Hamilton, 1967). Local Mate Competition of males for the access to females should occur when (1) males and females emerge simultaneously or when one sex wait for the other, (2) individuals do not need a sexual maturation and (3) when males and females emerge from the same local place (Hamilton, 1967). However, local mating between siblings should be avoided when the inbreeding consequences are too high compared to the probability to remain virgin after dispersal.

Aphid parasitoids, as many other Hymenoptera, share a Complementary Sex Determination rule that could render them very sensitive to inbreeding (Heimpel & De Boer, 2008). The sex is determined by the allelic composition at one or few loci, haploid individuals developing in males and heterozygote ones in females. When homozygote individuals are produced, they develop into diploid, often sterile, males (Heimpel & De Boer, 2008). Inbreeding sensitivity was observed in *Aphidus rhopalosiphi* (Salin *et al*., 2004) a close relative species of our model but no data are presently available in *A. matricariae*. However, no behavioural avoidance of sib-mating had been observed in this species (Bourdais & Hance, 2009), suggesting that other mechanisms of inbreeding avoidance should had probably been selected. The dispersal behaviour of aphids after the female parasitoid attack could thus favour outbreeding, promoting off-patch mating in this species. The patches of mummies obtained in this study contain only few individuals (between 2 and 10) that decrease the probability of mating on the patch if males and females emerge together. We observed in our cages that a mean of 60% of both sexes emerge alone on a turnip leaf because of the spatial position of mummies. This means that around 40% of males and females potentially emerge under a Local Mate Competition context, in very located areas. The observed sex ratio (45% of males) is in

accordance with a Partial Local Mating strategy with no direct inbreeding avoidance in the case of few females parasitizing the host patch (Hardy, 1994; Stubblefield & Seger, 1990). However, experiments were done under lab conditions, with standardized colony of similar quality aphids. Sex allocation strategies of females vary according to the patch quality, meaning the number and quality of hosts (i.e. the degree of parasitism) (Hardy, 1994). These aspects have to be explored to refine our understanding of the link between sex allocation strategies and Local Mate competition in aphid parasitoids. For instance, mummies spatial distribution under natural condition remains to be analysed in relation with their timing of emergence to improve our understanding of the probabilities of sib-mating and how sib-mating avoidance could had been selected.

One major future investigation concerns the definition of the word "local" in Local Mate Competition in aphid parasitoids. We have no idea of the male perception of the scale for mate searching. We can expect that a change from a walking behaviour on a leaf to the flight will correspond to a decision to leave the patch to explore a broader zone. In egg parasitoids with high level of Local Mate Competition, males optimize the time spent on the emergence patch (= clumped eggs) to maximize its fitness (Martel *et al*., 2008). In these species the natal patch is at the same spatial scale as the "mating patch". This strategy is optimal for short-lived species with low dispersal capacities such as Trichogrammatidae (Martel & Boivin, 2008) but the life history traits of Aphidiinae are different. Field and laboratory studies are now important to conduct to evaluate the spatial scale used by an aphid parasitoid male to mate after emergence and how it allocates time for searching for mates.

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3. Emergence kinetics of **A. matricariae** *and post emergence behaviour.*

Paper 2

Emergence rhythms favour outbreeding in the aphid parasitoid *Aphidius matricariae* (Hymenoptera: Braconidae).

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Submitted to Comptes Rendu de Biologie

Abstract

In insect parasitoids, mating strategies of males and females depend on both spatial and temporal emergence pattern of adults. Adults of quasi-gregarious parasitoids emerge from patchily distributed hosts and the rhythm of their emergence often favours sib mating on the emergence patch. However, in some inbreeding sensitive species, on patch mating should be counter selected and the emergence pattern of males and females could play a non-negligible role when no behavioural mechanism of sib mating avoidance exists in that species. To study the influence of emergence rhythms and protandry on the sibmating opportunities in a quasi-gregarious parasitoid, we observed the daily rhythmicity of emergence of a cohort of the aphid parasitoid *Aphidius matricariae* (Hymenoptera: Braconidae). It appeared that adult emergence is concentrated in the morning, males emerging in average before females, but with an overlap. A more precise evaluation of emergence pattern within a brood suggests that brother and sister rarely emerge in the same time and that the post emergence behaviour (dispersal) of males and females favour out patch mating. The relationships between rhythmicity of emergence, protandry and outbreeding in this species are thus discussed.

Key words

Mating strategies, aphid parasitoid, rhythms of emergence, protandry, inbreeding

Introduction

Organisms display a large number of behavioural, developmental and physiological events that are controlled by endogenous clock-like processes (Giebultowicz, 1999; Nunes & Saunders, 1999; Saunders, 2002). An array of daily behaviours such as general locomotor activity, flight, mating, oviposition, egg hatching, pupation and pupal eclosion are governed by well described circadian oscillators (Pittendrigh, 1967; Giebultowicz, 1999; Sakai & Ishida, 2001) while photoperiodic clocks mostly governed seasonal phenomena, such as the onset of insect diapause or larval growth rates (Tauber & Kyriacou, 2001; Saunders, 2009).

Insect parasitoids are organisms that develop in or on a host and kill it as a result of their development (Eggleton & Gaston, 1990). Thus, numerous parasitoid life history traits are strongly linked to those of their host. For instance, the mating system is influenced by the spatial and temporal distribution of adult emergence, which depends on the spatial distribution of the hosts and how the female laid its eggs inside the suitable host population (Godfray, 1994; Wandjberg, 2006). In solitary parasitoids that develop quasi-gregariously (i.e. when females parasitizes several hosts that are grouped) and gregarious parasitoids (i.e. when females laid several eggs in each solitary host), the newly emerged adults mate totally or partially on their natal patch. Local Mate Competition (Hamilton, 1967; Nunney & Luck, 1988) is thus the result of males and females developing on a common site and either emerging simultaneously from this site, either emerging asynchronously but remaining on the site until mating. The mating structure of these wasps is thus strongly influenced by several parameters such as male and female sexual maturation time, the resistance to inbreeding, the dispersal behaviour of both sexes but also the timing of adult emergences (Hamilton, 1967; Hardy, 1994; Fauvergue *et al.,* 1999; Greeff, 2002,). The study of emergence patterns thus provides a complementary but often neglected approach to mating strategies in parasitic wasps.

The daily pattern of adult emergence is reported in several parasitoids species and adults mostly emerge during the first hours of the day (Rounbehler & Ellington, 1973; Suzuki & Hiehata, 1985; Hirose *et al.,* 1988; van Lenteren *et al.,* 1992; Corrigan *et al*., 1995; Fanitou *et al.,* 1998; Fauvergue *et al.,* 1999; Mazzi *et al.,* 2011). In most of insects, including parasitoid species, protandry occurs, meaning that males emerge before females (e.g. Kainoh, 1986; Ruberson *et al*., 1988; Hasting, 1989; Wedell, 1992; Pompanon *et al.,* 1995; Morbey & Ydenberg, 2001; Doyon & Boivin 2005, 2006,). Different hypothesis about the evolutive significance of protandry in insect species had been suggested (Wiklund & Fagerstrom, 1977) but are still controversial (Morbey & Ydenberg, 2001).

In parasitoids, protandry is associated in quasi-gregarious and gregarious species with a mating system that maximizes the numbers of mating in males and minimizes the female pre-reproductive period (Pompanon *et al.,* 1995; Doyon & Boivin, 2006; Zonneveld & Metz, 1991; Bradshaw *et al.,* 1997). Under that hypothesis, female should be receptive to mating just after emergence and sib-mating should not have deleterious effects. Egg gregarious or quasi-gregarious parasitoids of the genus *Trichogramma* typically behave like this (Karpova, 2006; Doyon & Boivin, 2006). However, inbreeding is also known to have lethal consequences on various species of gregarious or quasi-gregarious hymenopterous parasitoids (Heimpel & DeBoer, 2008; Salin *et al.,* 2004). Indeed, inbreeding is critical in species with gender is determined by allelic composition at one or few loci where homozygote diploid individuals develop in sterile males (Heimpel & DeBoer, 2008). Under that context, another valuable hypothesis to explain protandry or differences in gender pattern of emergences in parasitoids could be linked to their inbreeding sensitivity. Protandry or protogyny associated with early dispersal should prevent from inbreeding if brother and sisters emerge in the same area and if no behavioural mechanism of sib-mating avoidance exists (Wiklund & Fagorstrom, 1977). However, despite of the importance of these aspects in quasi-gregarious and gregarious species the emergences patterns of both sexes and their post emergence dispersal was poorly studied (Doyon & Boivin, 2006; He & Wang, 2008).

The aim of this paper is to test the relationship between adult emergence rhythms in the quasi-gregarious parasitoid *Aphidius matricariae* (Hymenoptera, Braconidae), mating opportunities of both males and females and the avoidance of sib-mating on the natal patch. Species of the *Aphidius* genius are characterized by patchily distributed hosts (aphid colonies), a female biased sex-ratio (Hardy, 1994; He & Wang, 2008; Makaurer & Volkl, 2004), protandrous males (Verai, 1942; Mackauer & Stary, 1967; Giri *et al.,* 1982; Hagvar & Hofsvang, 1991; Brodeur & Rosenheim, 2000; Battaglia *et al.,* 2002; He *et al.,* 2004; He & Wang, 2008; Le Ralec *et al.,* 2010). Some evidences for inbreeding consequences had been found in *Aphidius* species (*A. rhopalosiphi*, Salin *et al.,* 2004), whereas they do not seems to avoid sib-mating by any direct behavioural decisions (*A. matricariae*, Bourdais & Hance, 2009). If male emerge and disperse before the emergence of females, few sib-mating will be possible on the emergence site. The alternative hypothesis could be that males and females emergence overlap within the whole population but that within a brood, the emergences of brother and sister are distributed so that their probabilities to mate on the patch just after emergence is low. In *Aphidius* species, very little precise quantitative information is available on adult emergence rhythms. The only study is on *Aphidus ervi* and suggests that males emerge in mean before females but that both sexes emergences overlap within a day (He *et al.,* 2004).

Because *Aphidius* species are known to be sensitive to inbreeding (Salin *et al.,* 2004), our main prediction is that the emergence rhythms decrease the sib-mating probability just after emergence, favouring dispersal of virgin individuals. To evaluate our predictions, we conducted the following experiments. (1) The daily pattern of emergences of both sexes in our laboratory control conditions of light has been described to evaluate if the species is protandrous or if males and females emergences overlap. (2) We then explored the effect of light on emergence rhythms using various photoperiodic regimes. This was conducted to verify if the onset could be perceived as a cue for synchronisation of emergence of males and females. (3) Female broods were used to observe if brother and sister emerge in close time intervals, favouring or not sib-mating probabilities on the natal patch. (4) In a last experiment, post-emergence behaviour was observed to evaluate the timing of dispersal of males and females from their natal patch. Knowing that no behavioural evidence of sibmating avoidance had been founded in this species (Bourdais & Hance, 2009) and some evidences of inbreeding lethal effects observed in close relative species (Salin *et al.,* 2004), we expected that the timing of males and female emergence do not favour sib mating on the natal patch.

Material & Methods

Biological material and experimental conditions

Aphidius matricariae (Hymenoptera: Braconidae) is a parasitoid wasp attacking a wide range of aphid species (Schlinger & Mackauer, 1963; Kalina & Stary, 1976). The parasitoids and aphids stains we used for this study were from Viridaxis S.A.©, which currently produce biocontrol agents. In our laboratory, *A. matricariae* was reared on peach potato aphids *Myzus persicae* (Hemiptera: Aphididae), which were raised on turnip plants (*Brassica rapa* L. subsp. *rapa*). Aphid and parasitoid cultures were maintained in separate rooms under identical standardized conditions (Temperature: 20±2C°, Relative humidity: 70±5% and Photoperiod: L16:D8).

Preliminary tests were conducted to evaluate the development time of our parasitoid stain under our laboratory conditions. The larval development spent 10 days before the formation of the mummy, which represents the beginning of the pupal stage of the parasitoid. Adult will begin to emerge 13 days after egg-laying. To obtain time-standardized individuals, we let 1 young (<24h) mated females parasitize 100 size-standardized aphids (i.e. aged of 2 days) during 2h in a Petri dish (Ø=9cm) (n=25). The limited period of oviposition of females was chosen to obtain individuals that were originated from eggs of the same age. After 10 days of development, every parasitoid pupa (i.e. mummy) was individually kept in 1.5 ml microcentrifuge tube.

Experiment 1. Temporal distribution of adult emergence.

To understand the temporal distribution of adult emergences of a cohort (25 broods of a single mated females that were allowed to parasitized a patch of 100 aphids during 2 hours), we use standardized mummies obtained in our control conditions of light (light from 7AM to 23PM). Because we had previously observed that emergence of the first parasitoids began after 13 days of development, we checked males and females emergences every 30min from 7AM to 23PM from that day 13 days post-oviposition) until the last day of emergence of the cohort. This allowed us to have data about the chronological pattern of emergence of both sexes and to test for protandry in this species.

The mean development time of males and females of the whole population was compared using Student's t-tests. The mean time of emergence between males and females from day to day was compared using a one-way Anova with day of emergence as a fixed parameter followed by Tukeys post hoc tests. The proportions of males and females that emerge between days were tested using a X^2 test.

Experiment 2. Influence of the onset.

To understand the influence of the lighting onset on adult emergence, we had to change the photoperiodic conditions of our rearing process to make possible the experiment. To do so, we reared 2 generations of parasitoids and aphids under a photoperiodic regimen of 12 hours of light and 12 hours of night (light from 9AM to 21PM). Then, we used standardized mummies obtain in the new conditions of light (light from 9AM to 21PM) that were randomly separated them in 3 groups. Each group was placed in 3 different photoperiod regimes from days 13 after oviposition: (1) control photoperiod with light from 9AM to 21PM, (2) advanced lighting (light from 8AM to 20PM) and (3) later lighting (from 10AM to 22PM). This method allows us to advance or delay the emergence of parasitoids from 1 hour only during the days of the insect emergences. From day 13 to day 15 post-oviposition we checked new males and females emergences every 30min during the entire photophase for each of the groups.

We analyzed the data using a mixed linear model (PROC MIXED, SAS Institute) that is designed explicitly for use with models containing both fixed (photoperiod, day, sex) and random effects (mother). The significance of fixed effects is tested with F tests that account for both the variance from the random effects and the error variance. We also wanted to have an idea of the kinetics of the emergence pattern and thus compare the different parameters of the kinetic curves.

Thus, the cumulative numbers of emerged parasitoids thought time were best fitted to a sigmoidal curve (GraphPad Prism, Copeland, 2000) using the formula $Y=M* X^h/(K' + X^h)$. In this formula, Y represents the value of the cumulative number of insect at day 'X', K' is related to the inflexion point (it is equal to the inflexion point when h=1), M is the maximum number of insects (plateau value) and h is the hill slope. F tests were used to compare the different parameters of the curves (GraphPad Software, Dan Diego, CA, USA, www.graphpad.com).

Experiment 3. Influence of the timing of emergence on sib-mating opportunities.

We let 19 females (mated, < 24h old) parasitize individually a patch of 100 size-standardized aphids during 2h in a Petri dish (Ø=9cm). After 10 days of development, every parasitoid pupa (i.e. mummies) from each female was individually kept in 1.5 ml microcentrifuge tubes until emergence. Adult emergences were observed under photoperiod light from 9AM to 21PM and the female origin of each individual was recorded.

To analyse our data, we first consider that the entire offspring produced by these 19 females represents what could happen in natural conditions under a high parasitism rate of numerous spatially closed aphid colonies. Two categories of individuals have been defined: (1) individuals that emerge without one of the opposite sex in a 30 minutes period of time and through the entire population (called "emergence with no partner") and (2) Individuals that not emerged alone in a 30 minutes period of time (defined as "emergence under on-patch mate competition"). The "emergence under on patch mate competition" individuals have been divided in three categories of potential competition: "kin" (only males and females of the same mother emerge in the same 30 minute period of time), "non kin" (no brother sister emerge in the same 30 minute period of time) or "kin + non kin" (if at least 1 brother-sister emerge together in the same 30 minute period of time). Comparisons between sexes were made using Chi square tests.

In a second analysis of the data we focussed on the female broods and compare the percentage of the brothers and sisters that emerge alone or under mate competition within a single brood (n=19). Comparisons between sexes were made using Chi square tests.

Experiment 4. Post emergence behaviour.

To evaluate the post emergence behaviour of males and females, we observe male and female behaviour after emergence of standardized mummies. Mummies were placed (groups of 40 mummies, n=5) on a Petri-dish (Ø45mm) that was placed in an other one (Ø90mm). Dispersal was effective when the parasitoid leave the central zone by flying or walking. Mummies were observed from 7AM to 15PM and emergences of individuals

observed. For each sexed individual, we noted the time of emergence, the time of dispersal from the patch and whether it was mated before dispersal (leaving the patch).

The proportions of individuals that disperse virgin *vs.* mated were compared using Chi-square tests. As dispersal time follows a normal distribution for males and females, we used a two ways ANOVA to test the effect of sex (with patch of emergence as a fixed parameter) on the mean dispersal time of individuals. T-tests were used to compare dispersal time between mated and unmated individuals. The percentage of male and females mate opportunity was calculated as the percentage of males and females that were present on the patch with at least one unmated individual of the opposite sex. Sex differences in the percentage of mate probability were compared using Chi square tests.

Results

Experiment 1. Temporal distribution of adult emergence.

The emergence of all individuals of the cohorts lasted 3 days and a total of 330 adults (127 males and 203 females) had emerged. The development time from oviposition to emergence was significantly shorter for males (18 837 ± 74 minutes) than that for females (19 283 ± 49 minutes) with a mean difference of around 8 hours (t-test, $t = 5.658$, $df = 328$, $p < 0.0001$). We did not observe any emergence during the night. Within a day, males and females began to emerge some minutes after the lighting onset (figure 1). Despite of a peak of emergences that occurred during the first hours after onset (especially in males), emergences of both sexes overlap during all the daylight (figure1).

The emergence patterns of males and females were different (figure 1, table 1). Most of the males (70% of all emerged males) had emerged on day 13 after egg-laying (table 1), with a peak just after the lighting onset (Figure 1). Within each day of emergence, most of the males emerge in a time interval of 4 hours after lighting (68.53% in day 13 and 80.1% in day 14). The emergence pattern of females was more complex. Females emerged at the same proportion on day 13 and 14 after egg-laying ($X^2 = 0.74$, $df = 1$, $p = 0.389$; table 1). Female emergences presented a peak during the morning of day 14 only (figure 1) while emergences in day 13 were concentrated during the afternoon (figure 1).

The mean hour of emergence after the lighting onset differ in males (207.6 ± 18.62, n=127) and females (338.30 ± 17.45, n=203) ($t = 4.923$, $df = 328$, $p < 0.0001$). However, it did not differ significantly from day to day in males but did in females (table 1).

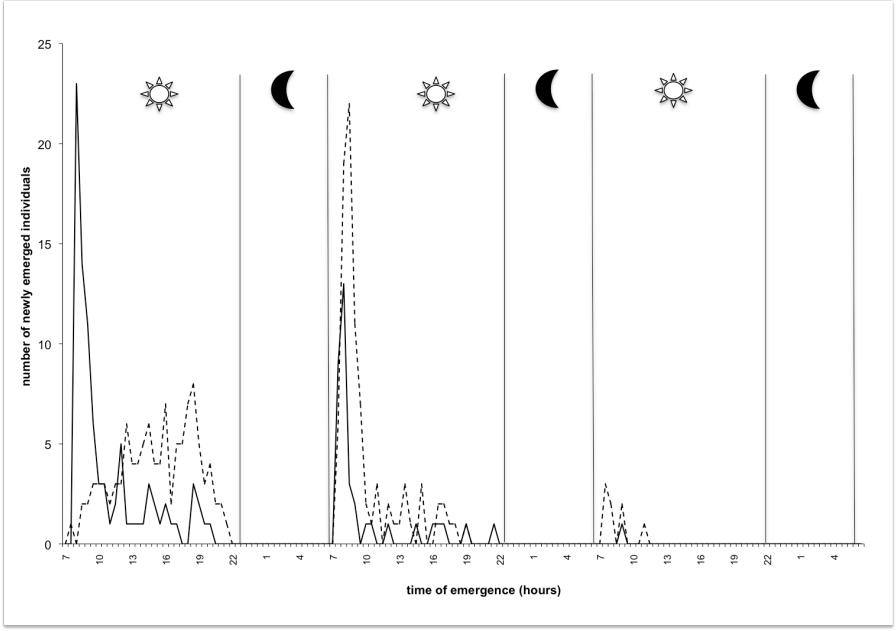

Figure 1. Distribution of males (dark lines, n = 127) and females (pointed lines, n = 203) adult emergences in *A. matricariae* under the standard photoperiodic regime (light dark photoperiod of 16:8, from 7AM to 23PM) Emergences are distributed over 3 days and are represented with the number of males and females newly emerged during each 30 minutes period of time.

Table 1. Pattern of adult emergence of *A. matricariae* males and females : repartition of emergence between days and mean time of adult emergence (number of minutes after lighting onset) within a day. Different letters indicate a significant difference between days for the same sex.

	Adult emergence percentage			**Adult emergence (Number of minutes after lighting onset, mean±s.e.)**		
Number of days after oviposition	13	14	15	13	14	15
Males	70.8 a	28.4 b	0.8 c	223.8±21.7 a	166.7±37.1 a	120 a
	χ^2=57.01, df=2, p<0.0001			ANOVA, F=1.125, df=2-124; p=0.3279		
Females	52.21 a	43.84 a	3.94 b	493.9±20.0 a	175.6±18.1 b	86.25±26.6 b
	χ^2=58.30, df=2, p<0.0001			ANOVA, F=76.86, df=2-200, p<0.0001		

Experiment 2. Influence of the lighting onset.

Effects of photoperiod, day and sex were all significant, but none of the interaction terms were (table 2). Within each day, males emerged in average earlier than females, with a mean time of male emergence of around 1h30 before females' for all photoperiodic regimes tested, but protandry was not always significant (table 2 and table 3). Time to emerge decreased from photoperiod 1 (7-19) to photoperiod 3 (9-21). Time to emerge was reduced for males compared with females. Time to emerge increased from day 1 to day 3 (table 2 and table 3).

Within each photoperiodic regime, the peak of emergence of males precedes the female one (see peak values, Table 4). The rate of emergence did not differ between males and females (see h parameter, table 4). Advanced or delayed photoperiod on the day of emergence advanced or delayed the pattern of 1 hour but did not change it for males (comparison of peak parameter, f tests, $F = 28.42$, $p > 0.05$) or for females (comparison of peak parameter, f tests, $F = 12.62$, $p > 0.05$).

Table 2. Mixed-model ANOVA for mean emergence time of males and females of *A. matricariae* under 3 different photoperiodic regimes.

Effet	**DDL Num.**	**DDL Res.**	**F**	**Pr > F**
Photoperiod	2	408	8,17	0,0003
Sex	1	408	20,52	<.0001
Day	2	408	4490,96	<.0001
Photoperiod*Sex	2	408	0,09	0,9171
Photoperiod*Day	4	408	1,14	0,338
Sex*Day	2	408	1,24	0,291
Photoperiod*Sex*Day	4	408	1	0,4087

Table 3. Mean time of adult emergence in males and females of *A. matricariae* under the three light dark photoperiods of 12:12. For each sex the mean time of emergence is given in minutes after onset ± SE. The mean differences between sexes within a day were tested using Student's t-test (Different letters in each raw indicate significant differences between days at p<0,05).

		Females	**Males**
Photoperiodic regime 1 (7AM-19PM)	Day 1	271 ± 29min (n=22) a	145 ± 22min (n=43) b
	Day 2	185 ± 24min (n=46) a	74 ± 15min (n=41) b
	Day 3	112.5 ± 33.4min (n=8) a	42 ± 12min (n=5) a
Photoperiodic regime 2 (8AM -20PM)	Day 1	307 ± 31.6min (n=42) a	169.2 ± 24.3min (n=64) b
	Day 2	172.5 ± 35.5min (n=20) a	100.8 ± 26.7min (n=25) a
	Day 3	64.3 ± 10min (n=7) a	30 ± 0min (n=2) a
Photoperiodic regime 3 (9AM-21PM)	Day 1	236.8 ± 31min (n=29) a	150 ± 28.4min (n=27) b
	Day 2	165 ± 25.7min (n=36) a	90 ± 27.3min (n=20) a
	Day 3	95 ± 9.2min (n=6) a	52.5 ± 14.7min (n=8) b

Experiment 3 - Inbreeding opportunities

Emergences of individuals of all the broods are distributed over 3 days, from day 13 to 15 after oviposition. Depending of the mother, the mean hour of emergence of males ranges from 45 minutes to 174 minutes after the lighting onset and from 83 minutes to 352 minutes for females. The between-mother variability is absent either for males and females (one-way ANOVA for males: f = 1.079, df = 18,194, p = 0.3763, for females f = 1.30, df = 18,184, p = 0.1918).

If we consider that the offspring of the 19 females represent a standardized field population, less males than females emerge with no partner presence (Figure 4a). However, none of all the males nor females that emerge in this population have to obligatory mate with a sib (figure 4a). 71.74% of males and 63.05% of females have no possibilities to mate with sib and finally for only 20% of males and 18% of females inbreeding is possible (figure 4a). If we supposed that an aphid colony was parasitized by only one female within a brief time intervall, around 80% of males and females emerge without any mate opportunity (Figure 4b, n =19 aphid colonies). The potential percentage of sib-mating on the natal patch is thus around 20% for both sexes (figure 4b).

Table 4. Paramters of kinetics of emergence of males and females *Aphidius matricariae* under the three photoperiodic regime. « Top » value represents the total number of emerged individuals, « peak » represents the moment (= mean±s.e. number of hour after onset) of the peak of emergence and « h » the value of the rate of emergence (mean±s.e. number of emerged individual per hour). The experimental data were fitted to a sigmoid curve by the method of the least squares ordinary fit (goodness of fit and the resulting values of p). Comparison between the cumulative numbers of emerged parasitoids in function of the treatment (photoperiod regimen). The parameters were compared two by two between males and females with F tests; the values of p are indicated.

☼ 8-20		Day 1		Day 2		Day 3	
		♀	♂	♀	♂	♀	♂
Best-fit values ± s.e.	Top	21.84±0.19	42.65±0.81	46.09±082	40.24±0.22	7.86±0.07	4.97±0.03
	Peak	4.63±0.07	1.50±0.21	2.36±018	0.69±0.03	1.16±0.05	0.38±0.07
	H	0.35±0.02	0.25±0.03	0.22±002	1.41±0.12	1.21±0.12	5.07±2.27
Goodness of Fit	DF	29.00	29.00	29.00	29.00	29.00	29.00
	R^2	0.99	0.91	0.95	0.98	0.97	0.96
Comparisons (F test, p value)	Top	P<0.001		P<0.001		P<0.001	
	Peak	P<0.001		P<0.001		P<0.001	
	H	NS		P<0.001		P<0.001	
☼ 9-21		Day 1		Day 2		Day 3	
		♀	♂	♀	♂	♀	♂
Best-fit values	Top	42.29±0.60	63.80±0.71	20.37±0.41	24.03±0.43	7.02 ± 0.03	2.00 ± 0
	Peak	3.72 ± 0.13	1.34 ± 0.12	1.83 ± 0.19	0.65 ± 0.15	0.84 ± 0.02	0.26 ± 0
	H	0.27 ± 0.02	0.28 ± 0.03	0.36 ± 0.06	0.61 ± 0.14	1.57 ± 0.11	60.43±0.3
Goodness of Fit	DF	29.00	29.00	29.00	29.00	29.00	29.00
	R^2	0.98	0.96	0.91	0.82	0.99	1.00
Comparisons (F test, p value)	Top	P<0.001		P<0.001		P<0.001	
	Peak	P<0.001		P<0.001		P<0.001	
	H	Ns		Ns		P<0.001	
☼ 10-22		Day 1		Day 2		Day 3	
		♀	♂	♀	♂	♀	♂
Best-fit values ± s.e.	Top	26.04 ± 0.36	24.85 ± 0.33	35.53 ± 0.61	24.45 ± 0.95	6.01 ± 0.01	7.91 ± 0.05
	Peak	3.05 ± 0.13	2.00 ± 0.13	1.91 ± 0.17	1.15 ± 0.47	1.35 ± 0.01	0.43 ± 0.03
	H	0.26 ± 0.02	0.27 ± 0.02	0.27 ± 0.03	0.15 ± 0.004	2.15 ± 0.06	3.13 ± 0.96
Goodness of Fit	DF	29.00	29.00	29.00	29.00	29.00	29.00
	R^2	0.97	0.96	0.94	0.78	1.00	0.96
Comparisons (F test, p value)	Top	P<0.05		P<0.001		P<0.001	
	Peak	P<0.001		Ns		P<0.001	
	H	Ns		P<0.05		Ns	

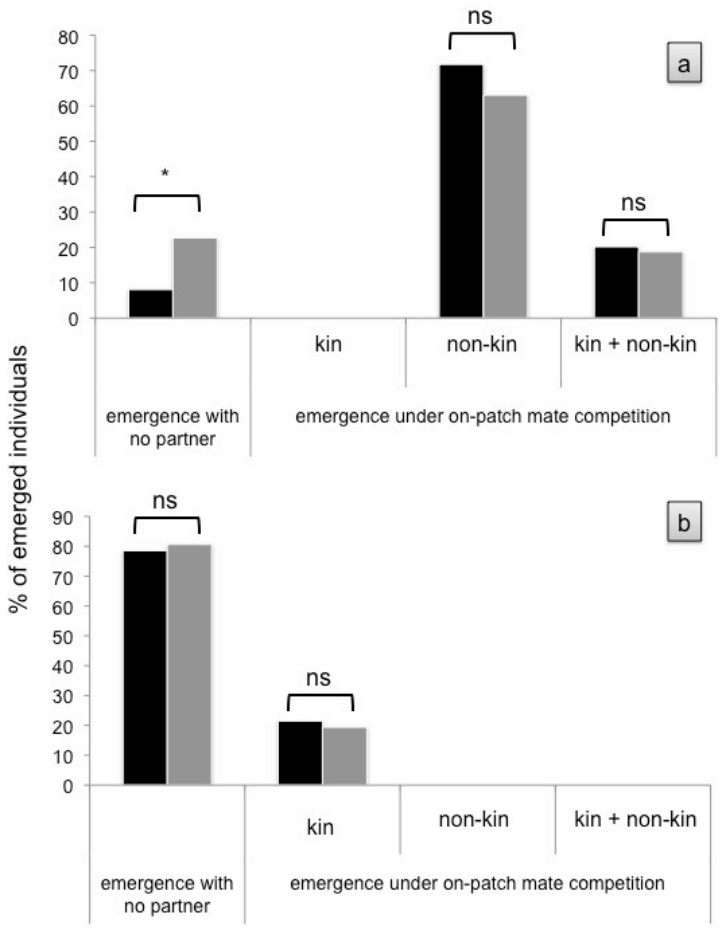

Figure 4. Proportion of males (dark bars) and females (grey bars) that emerge alone or under local mate competition and nature of the competition in relation to inbreeding. Fisher exact tests were done to compare males and females, * indicates a significant difference at $p<0.05$ (a) Data were obtained compiling the emergence time of the total offspring of the 19 females lines. (b) Data were analyzed for each mother offspring and given in mean percentage of emergence (n=19 broods).

Experiment 4 - Post emergence behaviour

We observed the post emergence behaviour of a total of 25 females and 63 males that had emerged from 5 different patches. The sex ratio of the patches was not different ($\chi^2=6.924$, df=4, p=0.139) with a mean proportion of males of 73.99%.

Females stay longer on the natal patch (1664.4 ± 153.4 seconds) than males (876.1 ± 60.2 seconds) before dispersing whatever the patch (ANOVA II: patch effect: p = 0.81, df = 4, f = 0.39, sex effect: p = 0.0011, df = 1, f = 11.49, interaction p = 0.66, df = 4, f = 0.60). Mated females do not disperse more rapidly than unmated ones (t-test, p = 0.13, t = 1.576, df = 19). However, unmated males disperse 793.2 ± 87.45 seconds after emergence (n = 58), that is more rapid than mated ones (1710 ± 146.6 seconds, n = 5) (t-test, p = 0.0036, t = 3.02, df = 61).

When we observed the percentage of males and females mate probabilities we found that 43.85% of males and 85.7% of females are present on the natal patch with at least one individual of the opposite sex, making mating possible more often for females than males (X^2=11.63, fd=1, p=0,0006). However, males under LMC more often leave the patch still virgin than females do (X^2=4.108, df=1, p=0.0427). We observed that 32% of females (n=8) and 11% of males (n=7) disperse from their natal patch mated.

Discussion

In the present study we analysed emergence rhythms of males and females of the aphid parasitoid *Aphidius matricariae* in regards to its mating strategies. Emergence rhythms have frequently been monitored in insect species (Saunders *et al.,* 2002; Wiklund & Fagerström, 1977; Zijlstra *et al.,* 2002; Zonneveld, 1996; Lankinen, 1986; Moller, 2004; Bradshaw *et al.,* 1997; Alcock, 1997) and in parasitic wasps (Morbey & Ydenberg, 2001; Kainoh, 1986; Ruberson *et al.,* 1988; Hasting, 1989; Wedell, 1992; Pompanon *et al.,* 1995; Doyon & Boivin, 2004; Fauvergue *et al.,* 1999; Mazzi *et al.,* 2011). Activities of insects are controlled by well known endogenous clocks modulated by exogenous factors (Saunders *et al.,* 2002) and are thus supposed to be adaptive (Morbey & Ydenberg, 2001). In quasi-gregarious species such as aphid parasitoid or egg parasitoids, one or few females, called the founderesses, often parasitize one patch of hosts within a brief period of time (Charnov, 1976; Tentelier *et al.,* 2006; Boivin *et al.,* 2004; Wajnberg, 2006). Adults of a same cohort are thus supposed to emerge from these hosts in a brief period of time (Beck, 1991; Sauders *et al.,* 2002). We effectively observed that rhythms of emergence of *A. matricariae* have similarities with those of the majority of other parasitoids: synchronisation emergences through an exogenous signal (light), protandry, and peak in the few hours following the sunset but the relation with their mating strategies have to be taken in account to understand the whole system.

Aphidius matricariae shows a typical emergence from pupae in morning hours as many diurnal insects with a clear maximum in the morning

and no emergence during the night (Lankinen, 1986; Ruberson *et al.,* 1988; Saunders *et al.,* 2002; Pompanon *et al*., 1995). Males emergences begin with light-on without any sign of anticipation at the darkness-light transition, in contrast with was observed in *Drosophila melanogaster* (Helfrish-Förster, 2001) but similar to most of parasitoid species (Pompanon *et al.,* 1995; Doyon & Boivin, 2006; Zaslavski *et al.,* 1999). When light-on was advanced or delayed from one hour, males and females wait for light to emerge, suggesting that ligh-on act as a signal to synchronize emergences. Nervertheless, the daily rythmicity of adult emergence restricted to certain times of the scotophase-photophase interface is known in several parasitoid species as in *Trichogramma semifumatum* (Rounbehler & Ellington, 1973) *T. minutum* (Corrigan *et al*., 1995), *T. embryophagum* (Krapova, 2006; Reznik *et al.,* 2008), *Nasonia* males (Bertossa *et al.,* 2010), *Aphidius ervi* (He *et al*., 2004). The emergence of parasitoids during the first hours after the onset probably coincides with more favourable conditions, as morning or night field temperatures are lower and humidity higher than during the mid day. The higher humidity rate in the morning induces lower water losses through the cuticle of newly emerged individuals (Lankinen, 1986) or allowed insects to spread their wings (Skopik & Pittendrigh, 1967). The emergence at the beginning of the daylight period had been suggested to favour reproductive or parasitism activity in parasitoids (Pompanon *et al.,* 1995; Karpova, 2006; Karpova & Reznik, 2002).

The variation between the peak delay between day 1 and 2 in females could reveal the expression of the moment of maturation of individuals. In most cases, eclosion from it own chorion is gated by the endogenous circadian system to a restricted period of the day (Myers, 2003) and individuals that reach maturity outside the gate will emerge from the host chorion at the next following gate, usually around 24h later. As males and females do not emerge during the night, individuals that are near to emerge in the afternoon perhaps ecolse but will wait for the morning to emerge (Myers, 2003; Bertossa *et al.,* 2010). Even if insect developmental rates and emergence rhythms tend to be similar for all individuals of a population reared under the same environmental conditions (Saunders *et al.,* 2002; Beck, 1991), variations in the developmental period of cohorts reared under the same conditions have been found in many insects (Shaffer, 1983; Doyon & Boivin, 2005) and could explain why we observed that the emergences of all individuals of a brood spread 3 consecutive days.

Aphidius matricariae males emerge often before females, but males and females emergences overlap so much within a population that we can not strictly validate the protandrous status of the species. In *Trichogramma evanescens*, males emerge before females in a real protandrous pattern (Doyon & Boivin, 2006) with at least no overlapping. The temporal patterns of adult emergence have been observed for some parasitoid species, mostly *Trichogramma* species (Reznik *et al.,* 2008) and are more or less

synchronized, depending of the species and of its mating strategy. Indeed, *Trichogramma* species are short-lived species (Boivin & Lagacé, 1999) for which dispersal capacities are quite low (Smith, 1996) and that mate mostly between relatives on the emergence patch (Martel & Boivin, 2004). In that case, the timing of emergence is very synchronized (Doyon & Boivin, 2006) to favour a rapid mate of both sexes before dispersal, as mating between sibs does not have lethal consequences. It seems that *A. matricariae* behave more like *A. ervi* (He *et al.,* 2004) or the gregarious larval parasitoid of the small white butterfly Pieris rapae, *Cotesia glomerata* (Mazzi *et al.,* 2011). In these 3 species, males and females emergences overlap even if in mean, males emerge before females. He *et al.* (2004) unfortunately did not discuss their result in term of mating strategies but Mazzi *et al.* (2011) interpreted the emergence pattern of *Cotesia glomerate* in function of the mating opportunities within a patch and the inbreeding costs. It appears that *Cotesia glomerata* do not suffer so much from inbreeding (Elias *et al.,* 2009) because inbred males are still able to mate and produce offspring contrary to many species with single-locus complementary sex determination (Heimpel & DeBoer, 2008). It is thus the post emergence behaviour of males that determine whether the significant difference in the timing of emergence of the two sexes for limits the occurrence of sibmating (Mazzi *et al.,* 2011). In *A. matricariae*, if we take in account the different females broods we observed that brother and sister rarely emerge together. The rhythms of emergence in *A. matricariae* appear to decrease the probability of mating with a sib on the natal patch and could have been selected to increase outbreeding. No behavioural mechanism of sib mating avoidance had been found in this species (Bourdais & Hance, 2009) even if mating with a sib could have deleterious effects on hymenoptera species with Complementary Sex Determination (Heimpel & DeBoer, 2008), including close relative species (Salin *et al.,* 2004). Our observations about the post emergence behaviour tend to favour this hypothesis because males disperse more rapidly than females and often disperse still virgin. Moreover, complementary experiments had shown that *A. matricariae* females need 30 minutes to be sexually mature and males do not need any sexual maturation (Bourdais, unpublished data). This could explain why few females disperse mated from the patch and why most of the males do not wait for females.

These results give some new indications about the mating strategies of the aphid parasitoid *Aphidius matricariae*. It seems that some particular strategies to avoid inbreeding had been selected in this species. (1) When a female lays eggs in an aphid colony, the offspring will emerge during 3 consecutive days, diluting the probabilities to mate with a sib on the natal patch. (2) Brother and sisters not emerge in a same period of time and thus have little chances to mate. (3) This is reinforced by the fact that males and females peak of emergence differ within a population by emergences overlap, making outbreeding more probable than inbreeding. (4) Finally the

tendency of males and females to disperse from the patch even unmated increases their probability to mate with a non-kin than with a brother. Thus, we supposed that encounter a sib on the emergence patch in nature is quite rare and that the importance of local mate competition is low (Hardy, 1994). We can imagine that emergence at the same time as conspecifics and/or sib could be possible only in cases of a high population level of aphids coupled with a high population level of parasitoids. However, mating systems of aphid parasitoids are quasi unknown especially regarding probability of mate encounters on the natal patch and future studies would benefit by considering for instance how aphids behave in their colony after being attacked by parasitoid females.

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4. Discussion

Knowledge of emergence patterns associated with a better understanding of mating system and oviposition strategies of the females are essential to better understand the ecology and evolution of reproductive strategies in parasitoid species. The main result of this part of the work is that the parasitoid *A. matricariae* is not as quasi-gregarious as suspected because of the dispersal of parasitized aphids. Our observations tend to confirm that some traits had evolved to decrease the inbreeding probabilities in this species.

Aphids disperse and parasitoids emerge alone

First, **we showed that aphids disperse** from the colony after an attack by *A. matricariae* females. Aphid modified behaviour has been reported in various studies using the aphid-parasitoid model, but the results have brought different conclusions in terms of evolutionary significance (Chow & Mackauer, 1999). These studies have suggested that dispersal of parasitized aphids was selected in order to reduce hyper-parasitism and predation, suggesting that dispersal would be adaptive for the parasitoid (Behrendt, 1968; 1971; Brodeur & McNeil, 1992). The important result for the present research is that aphids disperse from the "natal" colony (= the colony where they were at the moment of the parasitoid attack) when disturbed by the egg laying behaviour of the female parasitoid and that **mummies are usually not clumped** in the environment. This will favour a solitary emergence of parasitoids that could probably not find a mate just by walking in the vicinity but should have to fly and look for distant cues of mate presence. Thus, aphid parasitoids could not be considered as true quasi-gregarious species such as Trichogrammatidae are. However, when aphid colonies are big enough (300 aphids), the probability of clumped mummification of parasitized aphid increases, suggesting that aphid parasitoids can behave as quasi-gregarious species in some particular moments, even if in this case the size of the patches of mummies remains quite small (less than 10 mummies).

However, the perception by a parasitoid of what is a patch of mummies (see introduction part, "the patch problem") is still unknown. The transition between walking and flying behaviour can be used even if we do not know the distance of perception of pheromonal cues. It means however, that if some mummies are placed on two contiguous but different leaves, walking capacity of parasitoids by from one leaf to another should be tested.

Under field conditions because we have no idea of the mummy repartition on plants, aphid parasitoids are currently considered as quasi-gregarious species, but to my knowledge, no precise report of the observation of patches of mummies had been observed in field conditions. Personal observations support that in most of the case mummies are not clumped in the field but some cases a patch of mummies can be observed after a mass release in greenhouses (Salin, pers. com.) or in summer in cereal field (van Baaren, pers. com.). These observations underline the future importance of field studies to understand the mating strategies on aphid parasitoids in relation to the spatial distribution of mummies. Moreover, because aphid behaviour after an attack may vary between species, we should observe the localizations of mummies of *A. matricariae* using another aphid host, *Aphis fabae* or *Sitobion avenae* for instance.

Parasitoids kinetics of emergence favour outbreeding

We observed that emergences of individuals of the same brood are widely spaced in time (3 consecutive days of emergence) and that protandry globally exists within the same day even if males and females emergence pathways overlap. Moreover, males and females from the same mother rarely emerge together and therefore have little chance to mate.

It has been known for a long time that the patterns of activity in insects are rhythmic (Saunders, 2002) and it is the same for emergence. The results of this study show that males and females of *A. matricariae* present a peak of emergence in the early hours of the photophase, suggesting that the arrival of light would act as a signal synchronizing the function of emergence. This effect of the arrival of the day has already been shown in some species such as *Aphidius ervi* (He et *al.,* 2004), or *Trichogramma minutum* (Martel & Boivin, 2004). Some authors have suggested that the emergence synchronized with the start of photophase coinciding with better environmental conditions for mate or hosts searching behaviours, or reducing the risk of predation (Lankinen, 1986; Pompanon *et al.,* 1995; Fanitou *et al.,* 1998). This have to be tested in our model but this explanation probably not explained the entire pattern because we do not observe a real peak as in other species such as in *Trichogramma*. Even if most of individuals emerge in the morning, a significant part of them emerge during the afternoon (around 50% of females in day 1, see results in paper 2).

Another interesting observation is the similar emergence pattern of females on day 2 (see paper 2) than males on day 1. This argues the fact that females are probably mature on day 1 but wait some hours for more optimal conditions to emerge. We know by measurements that there is no difference in the size of females emerging on day 1 or waiting for day 2 (see annex 1). Females that wait in the host chorion can thus have time to be sexually

mature when they emerge but waiting could also have costs because they cannot feed and are more vulnerable to predator. Comparisons of sexual maturation time and fitness of these two kinds of females should now be done.

There are various explanations for **why males emerge before females** but most of them seem non relevant for our model. Wiklund (1977) hypothesized that protandry results of intraspecific competition between males for females and would be the optimal reproductive strategy in species where the females are monoandrous or in which mating with virgin females increases the fitness of the male. In all cases, emerging before females gives time for males to search for receptive females on the patch, favouring males that emerged earlier. Especially if the light is the signal, all the females will begin their emergence at the same time in the same environment and so even if males disperse from their natal patch, they can find newly emerged females. This hypothesis could not fit with our model, mainly because of the aphid dispersal and because mummies are not clumped, but also because the peak of emergence is too large within a day.

Another possibility is that protandry is the result of sexual dimorphism. Males emerge earlier because they are smaller than females (Mackauer 1996). However, even if the males of *Aphidius matricariae* are generally smaller than females, no relationship between size and emergence time or duration of development has been highlighted (see annex 1).

Thus, the potential explanation of such a pattern of emergence (protandry in mean but overlapping of male and females emergences, no sister and brother emergence if the same period of time) could be linked to the inbreeding avoidance. If no behavioural mechanisms exist in the species to avoid mating between sibs, such a mechanism should act to decrease the probability to brother-sister mating on the natal patch. This aspect will be discussed in more details in the final discussion, in the lights of the results obtain in mate choice under sibmating opportunities (paper 5).

Finally, **post-emergence behaviour** of both sexes suggests that individuals disperse fairly quickly after their emergence and that mating on the natal patch seems uncommon. In our experiments, males disperse mostly un-mated, while more mated females disperse. However, again, this is probably very rare in nature because of the patterns of dispersal of parasitized aphids that make the parasitoid mummify alone and not in patches of mummies. No data is available on the post emergence behaviour of aphid parasitoids except in our experiments, despite of its importance in understanding the mating strategies. In *Trichogramma sp*., that are quasi-gregarious species, some studies have shown that emergences are very synchronized (Martel & Boivin, 2004) and that males disperse more slowly than females (Martel & Boivin, 2004; Forsse *et al.,* 1992), because they wait

the emergence of females on the natal patch. However, some variations between species exist because males *T. pintoi* begin to disperse quite quickly, which could also promote mating with females emerged on another patch (Nunney & Luck, 1988) and thus promote genetic exchange between subpopulations. In these species, the adult dispersion would be primarily driven by the reproductive status of females (Pompanon *et al.,* 1995), and mated females disperse more quickly. This post-emergence behaviour could also be a mechanism of decreasing the Local Mate Competition, but this has to be tested.

More precise investigations are now needed to understand what can motive both sexes to disperse from their natal patch or stay and wait. In females, the presence of aphids near the natal patch could act as a motivation to stay and began to lay eggs without been fertilized (and produce only males) while males can wait for females if they have the possibility to evaluate the time they have to spent on the patch before the female emergence. This implies that they can recognize the sex of the future adult inside the mummy and adjust their behaviour in accordance to it. Personal observations could support this hypothesis because males sometimes show the wing fanning behaviour towards mummy but we did not quantify it more precisely.

Influence of aphid dispersal behaviour on the mating system of its parasitoid and consequences at a population level

Aphid dispersal behaviours favour the solitary emergence of males and females when aphid populations are low. By increasing the density of aphids, we observed that patches of mummies occurred more often, probably simply as a result of the increasing density of the population in lab conditions (cages where no escape is possible). Moreover, our experiments on the emergence rhythms of parasitoids and their post-emergence behaviour had shown that males and females emergences overlap within a population (around 400 mummies in emergence experiment) but that the dispersal behaviour of adults make on-patch mating rare, even in case of big mummy patches (40 synchronized mummies).

Thus, the emergence pattern imposed by aphid dispersal probably acts to increase the genetic diversity of the species, or at least avoiding the genetic depression, by prevent brother-sister mating. Experiments using different aphid colonies and taking in account a larger scale are now important to conduct. Indeed, if few females parasitize aphid colonies that are near each other (less than 50cm, the distance of aphid dispersal of our experimental cages), aphids will disperse and will potentially mummified by chance next to one mummy originated from the neighbour aphid colony. In that case, males and females that emerge together have proportionally lower

chances to be related. In the same approach, we have to conduct more precise experiment on the dispersal capacities of males foraging for females to evaluate what is the scale of a patch for a male.

Main conclusions of this chapter

In conclusion, different mechanisms have been selected in *A. matricariae* to reduce the probability of meeting and mating on the patch of emergence. In the particular case of *A. matricariae*, and probably in other aphid parasitoids, it seems that the **adult emerges more like a solitary species** than a quasi-gregarious one, even if a quasi-gregarious type of emergence pattern could happen in particular conditions (high level of host population coupled with high level of parasitism of aphid colonies).

Life history strategies of the species would be better fit with a "Partial Mate Competition" type with low levels of on-patch mating, and has favoured the selection of various traits to easily find a mate out of the patch, such as the use of pheromones, a long mating window of mating for both sexes or a low sexual maturation time. In a parallel way, if emergence patterns (both in space and time) of brothers and sisters seem to act together for avoiding sib-mating, no direct behavioural avoidance should be selected in the species.

Chapter IV. Inbreeding avoidance mechanisms acting during pair formation

1. General introduction

It appears that *A. matricariae* is probably more a solitary species than a real quasi-gregarious one when we observe the repartition of mummies in our experimental cages and the patterns of emergence of adults (see the precedent chapter). If males and females emerge alone in the environment, or at least without easy-finding partners next to their natal point, some behavioural and physiological traits should have been selected to favour male-female encounters and mating, but also to favour outbreeding.

If males and females emerge alone in the environment, the **sex ratio** should be near the equilibrium of 50% of males, as in solitary species and according to Fisher theory (Fisher, 1930). However, things are a little bit different in haplo-diploid species. In that particular case, it is advantageous for a female to produce more daughters than sons. Theory says that in quasi-gregarious species and gregarious ones, brothers compete for a limited number of females to mate, through a process called Local Mate Competition (LMC: Hamilton, 1967; Antolin, 1993). The model predicts that the proportion of males should decrease with increasing LMC. Some models show that both male dispersal and a high number of foundress females lessen the competitive asymmetry between sons and daughters and diminish the sex ratio bias towards females. In haplodiploid species, inbreeding also selects a female-biased sex ratio (Fauvergue *et al.,* 1999). However, as aphids disperse and favour a solitary emergence of parasitoids, the level of LMC should be lower and the sex-ratio should be near the equilibrium.

Secondly we can suggest that males and females use **kairomones and/or pheromones** to locate potential mates. We know that *Aphidius* females are attracted by kairomones (aphid odours or infested plant odours). Some studies on *A. ervi* females had shown that they use cornicle secretions of the pea aphid *A. pisum* as a kairomone (Battaglia *et al.,* 1993; Battaglia *et al.,* 2000). They also use odours from plant or infested plant to orientate them in the environment (Du *et al.,* 1996). A study on *Aphidius colemani* showed that males also used plant odour to orient themselves in the environment (Douloumpaka *et al.,* 2003) and *A. ervi* males can be conditioned to vanilla odour if this odour is associated with mating (Villagra *et al.,* 2005). Male insects are known to respond to female's pheromones. We know that mating is modulated by two pheromones: one that stimulates male upwind flight and a short distance one that stimulates courtship behaviour (McNeil & Brodeur, 1995; Marchand & McNeil, 2000). However, there are not so many studies on sexual pheromones in parasitoids and even

less where the sex pheromone has been identified (Ellers *et al.,* 1984; Kainoh *et al.,* 1991; Swedenborg & Jones, 1992). In aphid parasitoids, sexual pheromones were well studied in *A. nigripes* (McNeil & McClure, 1995) or in *A. ervi* (McClure *et al*., 2007) but never in *A. matricariae*.

In this thesis, we did not conduct experiments about sex ratios nor pheromonal or kairomonal use of males and females to find a mate. However, some of our observations will be discussed at the end of this chapter.

Then, physiological traits should favour mating during a large period of time because some females and males potentially stay virgin for long periods. However, a **short sexual maturation time** should have been selected because if mummies are clumped (in case of an important aphid colony or important populations of aphid within a landscape), males and females will benefit from mating as soon as they can. As reviewed in the introduction part about mating systems in parasitoids, few studies explored the sexual maturation time in aphid parasitoids and often males and females used in experiments were just "24h old". It appears to be necessary to study it in our model. A **long period of being receptive to mating** should also have been selected. If males and females are very dispersed in the environment, they should mate even if they are old. It is especially the case for males that are selected to compete for the access to a maximum of females. Moreover, a **different sexual maturation time** between males and female should had been selected to avoid mating between sibling in the case of a simultaneous emergence of males and females on a patch of mummies.

Polyandry should also be interesting in case of solitary emergence of females. By being polyandrous, females can mate with every male they found and use cryptic choice to favour one male than one another. However, monoandry is more often founded in solitary species and polyandry in quasi-gregarious or gregarious ones (Ridley, 1993). Thus, according to the literature, our species should better be monoandrous than polyandrous. If monoandry is the rule, males should not loose time to court mated females, the signal that make them unreceptive should be clear. This is supported by a study on *A. nigripes* that showed a significant and permanent reduction in pheromone production after mating (McNeil & Brodeur, 1995). However, the alternative hypothesis is that monoandry could be sufficient in this species to optimize the female fitness and that the selective forces against inbreeding are not enough important to favour polyandry to dilute the effect of inbreeding.

Solitary emergence reduces the pressure on **sib-mating** avoidance because the probability to encounter sib is rarer than when mating occurs on the natal patch just after emergence. Sib-mating avoidance can appear in case of gregarious or quasi-gregarious species if mating on the emergence

patch is common and if inbreeding has deleterious effects on the offspring. It has been showed that for some hymenopteran species sib-mating have negative effects because of the sex determination rules (Salin *et al.,* 2004) but we presently have no data on mate choice in relation to inbreeding in aphid parasitoids. We predict here that no direct behavioural avoidance of brother-sister mating exist in *A. matricariae*, mostly because of the spatio-temporal characteristics of their emergence.

To summarize, we conducted 3 major experiments to better understand the mating system of *A. matricariae* and how some physiological or behavioural traits could act to favour inbreeding avoidance.

(1) First we wanted to know if *A. matricariae* females were really monoandrous as cited in the literature but never proved. During this experiment we tested two main hypotheses that could favour polyandry in insect females: the sperm depletion in quality because of a long storage in the spermatheca of the female and the sperm depletion in quantity because of the oviposition behaviour of the female. This experiment also allowed us to verify the controversial result of Verai (1942) who found that *A. matricariae* females reject any mate if they had begun to lay eggs, even if virgin.

(2) Secondly, we wanted to observe the sexual maturation time in both sexes and observe if mate choice in function to the age of both sexes happen. We also conducted experiments about the mating window of both sexes.

(3) Finally, we observed if males and females can avoid sib-mating by behavioural changes when brothers and sisters are put together and in cases of mate choice.

2. Number of mate that **Aphidius matricariae** *female accepts. Is she really monoandrous?*

Shift in mating strategy with oviposition in *A. matricariae* females

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Abstract

Understanding the mating rate of females is essential to understand mating strategies of a species. Indeed, being monoandrous or polyandrous has important consequences on both pre-copulatory and post-copulatory strategies of males and females. In this work we explored the female mating frequency of the aphid parasitoid *Aphidius matricariae* (Hymenoptera: Braconidae). We first tested if there is a relation between the percentage of polyandry and the time elapsed between two mating opportunities without egg-laying. We found that a maximum of 10% of the females mate twice, suggesting that a low proportion of females can be polyandrous under lab conditions. In the second experiment, newly emerged females were mated and were daily allowed to lay eggs and to re-mate during the rest of their life. Here we did not observe any re-mating, although 36% of the tested females were sperm depleted (production of 100% males before death). Hence, this suggests that females behave and accept mating differently if they have access to hosts after the first mate or not. The percentage of polyandrous females increases with time in absence of host. In the same time, the sex ratio of the progeny of females with access to hosts is increasingly male biased when time since last mating increases. These two results suggest that in cases of host absence, mated females tend to re-mate with time since mating, probably because of a decrease in sperm quality when stored in spermatheca

Keywords

Monoandry – aphid parasitoid – polyandry – egg laying – mate number

Introduction

The evolution of mating rates of males and females is a cornerstone of reproductive biology and has generated an extensive theoretical and empirical literature in insects (Arnqvist & Nilsson, 2000; Hosken & Stockley, 2003; Hosken *et al.,* 2009). Understanding the mating rate of females is of importance because the selection of monoandry or polyandry has important consequences on the selective pressures linked to the mating strategies. Although many of the direct and indirect costs and benefits of mating to females are now well documented (Arnqvist & Nilsson, 2000; Jennions & Petrie, 2000; Wiklund *et al.,* 2001; Shuker & Day, 2002; Arnqvist & Rowe, 2005; South & Lewis, 2011), our understanding of the evolution of polyandry is incomplete. For example, it is still not clear if and in what sense female mating rates are optimal (Arnqvist & Nilsson, 2000), or whether females are constrained (Wiklund *et al.,* 2001) or manipulated by males (Holland & Rice, 1998) into mating sub-optimally. Indeed, the male point of view, female polygyny decreases their probability of a successful sperm transfer by increasing the sperm competition through direct mechanisms or cryptic female choice (Parker, 1979; Eberhard, 1996). Males of many species have thus developed various strategies to ensure their sperm monopoly and optimize mating (Eberhard, 1996; Engqvist & Sauer, 2001). For the female, polyandry may also depend on the availability of non-kin males and of possibility to lay eggs on the appropriate resource. Indeed, in absence of resource for its offspring a female may refrain egg laying which may result in a progressive decrease of sperm quality. In that case, a second mating may be expected. It could be particularly the case for insect parasitoids where female fecundity depends on host availability.

Parasitoid wasps are a large group of Hymenoptera insects whose larvae develop by feeding on the bodies of others organisms (Godfray, 1994; Boivin *et al.,* 2012). In solitary species, one individual emerge from each host while in gregarious species numerous individuals will emerge per host. Quasi-gregarious parasitoids correspond to an intermediate state where one individual emerge per host but the hosts are clumped and several hosts may be parasitized in the same aggregate (van den Assem *et al.,* 1980). In a meta-analysis of 97 species of Hymenoptera parasitoids, Ridley (1993) found a correlation between the solitary or gregarious status of parasitoids and their mating frequency. He observed that solitary species tend to be monoandrous while gregarious ones tend to be polyandrous. He argued that the advantage of genetically heterogeneous offspring could be greatest among gregarious species, because siblings develop in the same host and compete together for resources. On the other hand, a further, non-exclusive circumstance that may favour polyandry could be he variance of the insemination capacity of the gregarious polygynous males, because sperm transfer can decay after multiple mating (Damiens & Boivin, 2005) and females face the risk

receiving insufficient sperm or no sperm. As quasi-gregarious parasitoids often show a female biased offspring sex ratio as gregarious ones, Godfray (1994) predict polyandry for quasi-gregarious wasps also. However, the question is still debated as literature reports monoandry for some quasi-gregarious species, such as *Spalangia endius* (Hymenoptera, Pteromalidae) (King *et al.,* 2005), *Pachycrepoideus vindemiae* (Hymenoptera, Pteromalidae) (Nadel & Luck, 1985) and polyandry for others such as *Trissolcus basalis* (Hymenoptera, Scelionodae) (Loch & Walter, 2002) or other Braconidae species (Kazmer & Luck, 1991; Pintureau *et al*., 1997; Chevrier & Bressac, 2002; Jacob & Boivin, 2005). Moreover, in some apparently quasi-gregarious monoandrous species, it appears that double mating may occur within a short time interval after the first mating (Khanh *et al.,* 2005; Mc Clure *et al.,* 2007; He & Wang, 2008). Our understanding of the quasi-gregarious species female mating strategy is clearly incomplete. In inbreeding sensitive species such as *Aphidius* ones (Salin *et al.,* 2004), where no direct behavioural kin avoidance exists (Bourdais & Hance, 2009), polyandry could be one of the mechanisms used to decrease the impact of sib-mating.

This study analyses the potentiality of multiple mating for females of the aphid parasitoid *Aphidius matricariae*. We tested the influence of the time elapsed between 2 mate proposals with and without egg laying on the propensity of females to re-mate. While, it has been shown for some closely related species (Subba Rao & Sharma, 1962) and even in *A. matricariae* (Verai, 1942) that virgin females ignore males after the initiation of oviposition, suggesting that oviposition could act as a chastity belt. Alternatively, if females are mated, the sperm use due to egg laying and egg fertilisation could lead to a need for new sperm and favour polyandry (Arnqvist & Nilsson, 2000).

Material & methods

Study organism and rearing.

In the laboratory, *Aphidius matricariae* was reared on the peach potato aphid *Myzus persicae*, maintained on turnip plants. Insect colonies were established by using material provided by Viridaxis S.A. (Belgium) that uses an Italian strain for commercial mass rearing. Aphid and parasitoid cultures were maintained in separate rooms, both under 20 ± 2°C, 70 ± 5% r.h. and L16:D8. To standardise parasitoids in size and thus avoid any effect of size on mate acceptance, newly emerged mated *A. matricariae* females were isolated and allowed to parasitize a maximum of 100 2nd stage aphids (aphids aged between 2 and 4 days) placed in a Petri dish (Ø = 9 cm) during 4 h. Then, to obtain naïve individuals for bioassays, all pupae (i.e. mummies) produced by these females were kept individually in 1.5 ml microcentrifuge

tubes until emergence. The mummies were checked twice daily and we used only males and females that emerged in the morning, between 7 a.m. and 1 p.m.

Mating experiments.

The experimental set-up is reviewed in figure 1.

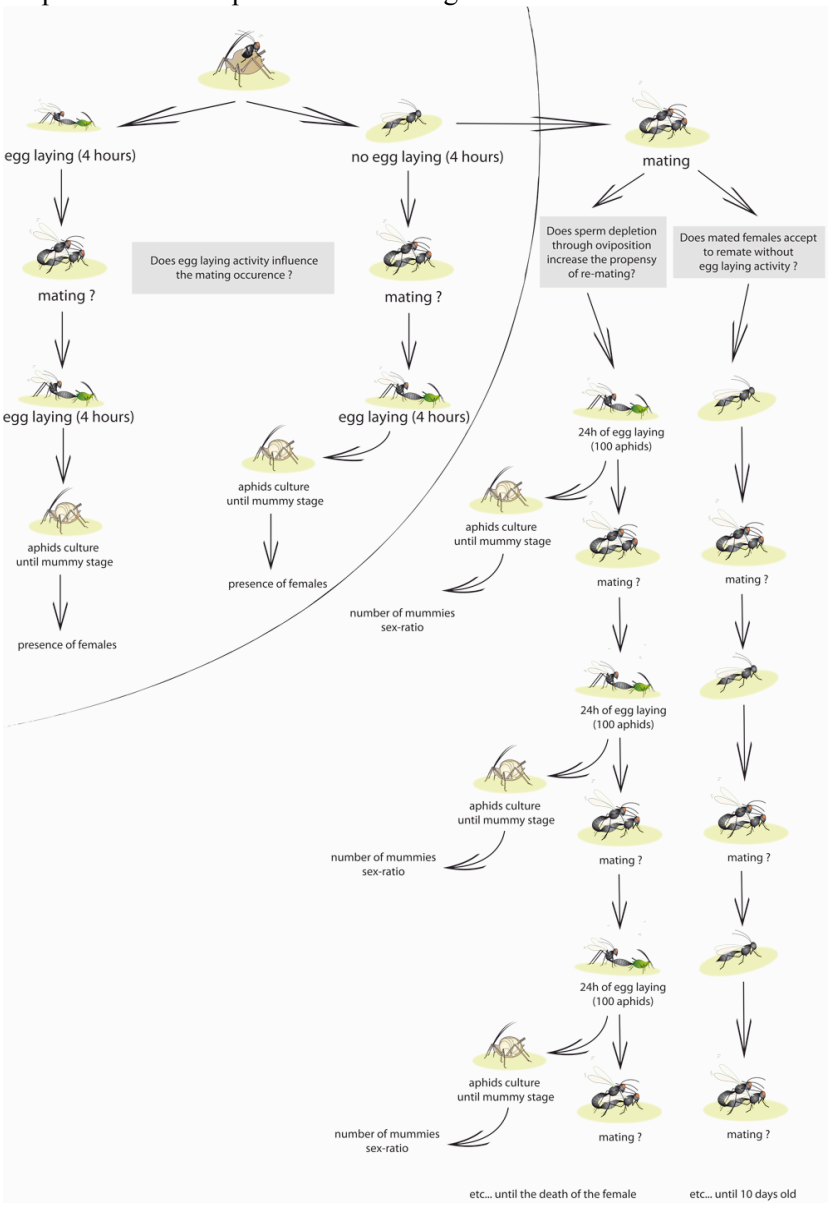

Figure 1. Experimental set-up showing the 3 different experiments performed.

Does egg-laying activity when virgin influence the mating occurrence?

Because egg-laying could affect virgin female mating acceptance rate, the propensity of virgin females of *A. matricariae* that had begun to produce offspring that accepted mating has been studied. Virgin young receptive females (< 6 h old) were put in the presence of 100 size-standardised aphids during 4 hours in a Petri dish (Ø = 9 cm) before presenting a potential mate. One naïve virgin male (< 6h old) was placed in a clean 1.5 ml microcentrifuge tube with the female for no longer than 5 minutes (sufficient period to mate if both sexes are receptive, pers. obs.). Mating was observed (n = 41) and compared with mating rate of females that did not have access to hosts (n = 43). The mated females were removed from the tube directly after mating before being placed in the presence of 100 size-standardised aphids during 4 hours in a Petri dish (Ø = 9 cm). We reared all aphids until the emergence of parasitoid offspring and recorded if daughters were present in the offspring.

Do mated females accept re-mating without any egg-laying activity?

One naïve virgin male (< 6 h old) was placed in a clean 1.5 ml microcentrifuge tube with one naïve (i.e. no access to aphids before mating) virgin young female (< 6 h old) for no longer than 5 minutes. Mating was observed and the mated female was immediately removed from the tube. She was directly presented to one other naïve virgin male (which represents 0 days between 2 mate proposals) or placed in a Petri dish (Ø = 9 cm) with water and honey until the second mating opportunity (1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days or 11 days after the first mating day). Re-mating occurrences were recorded. At least 60 different females were used for each interval of time between two matings. We choose these durations because even if females can live in lab conditions more than 11 days, virgin females of 11 days old and more are unattractive to young naïve males (Bourdais & Hance, unpublished data).

Does oviposition increase the propensity of re-mating?

We then tested if oviposition will stimulate a female to accept a second mate. As all Hymenoptera species, *A. matricariae* produce males by laying unfertilised eggs and females by laying fertilised eggs (Heimpel & De Boer, 2008). Because more than one spermatozoid is often needed to fertilise an egg (Sasaki & Obara 1999; Henter 2004), egg-laying will result in progressive sperm depletion. A female depleted of sperm in spermatheca will thus produce 100% male offspring. However, because host quality can change the sex allocation in parasitoids (Godfray, 1994), we provide optimal conditions to females (good and constant host quality, food and water). In a first step, twenty-two newly emerged and mated females were left in the

presence of a patch of 100 size-standardised aphids during 24 h to allow maximum parasitism per day. This was repeated from the day of emergence until the day of death of the female. Aphids from each 24 h egg-laying period were reared until the emergence of the parasitoid to check for offspring production (number of mummified aphids) and the evolution of sex ratio (here defined as the percentage of males).

To estimate the effect of sperm depletion of re-mating propensity, these twenty-two newly emerged females were presented to a male after each of the egg-laying sessions described above. To do so, after a 24 h period of egg-laying and every 24 h periods until the death of the female, each female was put in a clean 1.5 ml microcentrifuge tube with one naïve virgin male for no longer than 5 minutes in order to check if the female accepted a second mate or not.

Statistical analysis.

Proportion of virgin females that accept to mate after egg-laying (experiment 1) and the proportion of females that accept to remate (experiment 2) was tested using Chi-square tests. The evolution of non egg-laying female that accept to remate was adjusted to a normal function model (GraphPad software v5.1, Dan Diego, CA, USA, www.graphpad.com).

We tested the influence of time and female on the probability to produce a male using generalized linear model (distribution: binomial, link: logit) implemented in SAS. Then we replaced the female effect by life duration. We selected the best model according to the AIC criterion (Burnham & Anderson 1998). Simple linear regression was used to model the potential relationships between the female lifespan and the total number of mummy they had produced. Paired t-tests were used to analyse the difference between the number of mummies produced and the proportion of males between the first and the last egg-laying period of females.

Results

Does egg-laying activity when virgin influence the mating occurrence?

The acceptance percentage for a first mating for *A. matricariae* females was not influenced by egg-laying (χ^2 = 0.663, p = 0.41), with respectively 93% (no egg-laying period as a virgin: n = 43) and 87% (egg-laying period as a virgin: n = 41) of mating acceptance. Because of experimental problems, the precise proportion of males and females in the offspring was not recorded. However, all observed matings were effective because of the presence of female offspring in 100% of the tested females.

Do mated females accept re-mating without any egg-laying activity?

A maximum of 10% of *A. matricariae* females have accepted a second mating when they have no access to hosts between two mating opportunities. This proportion increased with the time interval between two mate propositions for females less than 6 days old, and then decreased (Figure 2). The link between the time between two mating (days) and the proportion of females accepting a second mate followed a normal function described as: $Y=10.15*\exp(-0.5*((X-5.162)/3.017)^{\wedge 2})$ where y = time between two mating and x = the proportion of females accepting a second mate (DF = 9, $R^2 = 0.96$, Absolute Sum of Squares = 4.938).

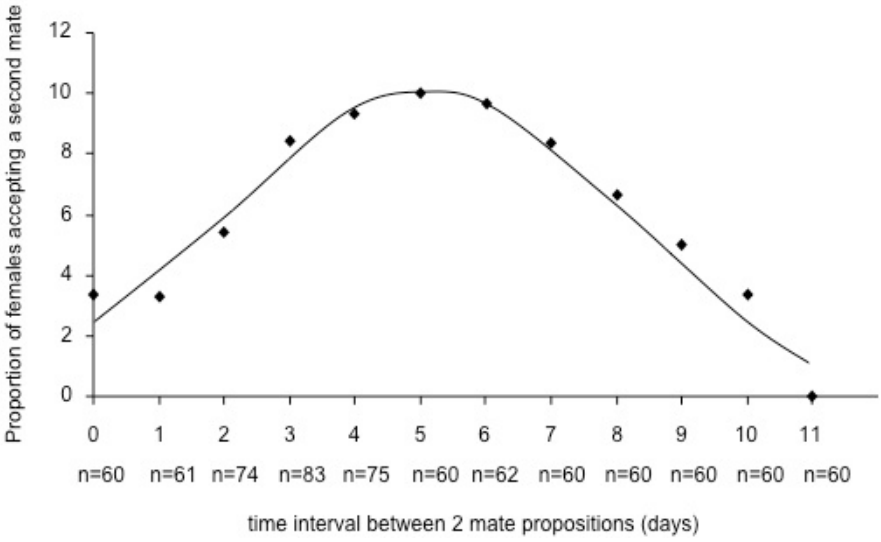

Figure 2. Proportions of naïve (i.e. not had access to hosts) mated females of *Aphidius matricariae* accepting a second mating in function of time elapsed between two mate proposals (days). 0 on the X axis corresponds to a second mating just after the first one. Between 60 and 83 females were used for each modality.

Does oviposition increase the propensity of re-mating?

Females that were allowed to parasitize aphids lived between 3 and 17 days (median age of 6 day, range = 3-9.2, n = 22) and produced between 46 and 419 offspring (mean±s.e.=156±19.5, n = 22) with a mean of 28.15±1.65 offspring per 24h (n = 147 egg-laying periods). There was an influence of the female longevity on mummy production: females that live longer produced more mummies (figure 3). The link between the female longevity (days) and the total number of mummies produced by these females could be expressed by the relationship y = 22.67x + 39.7 with y =

longevity and x = total number of mummies (n = 22, $r^2 = 0.71$, Pearson coef. = 0.84, $p < 0.0001$).

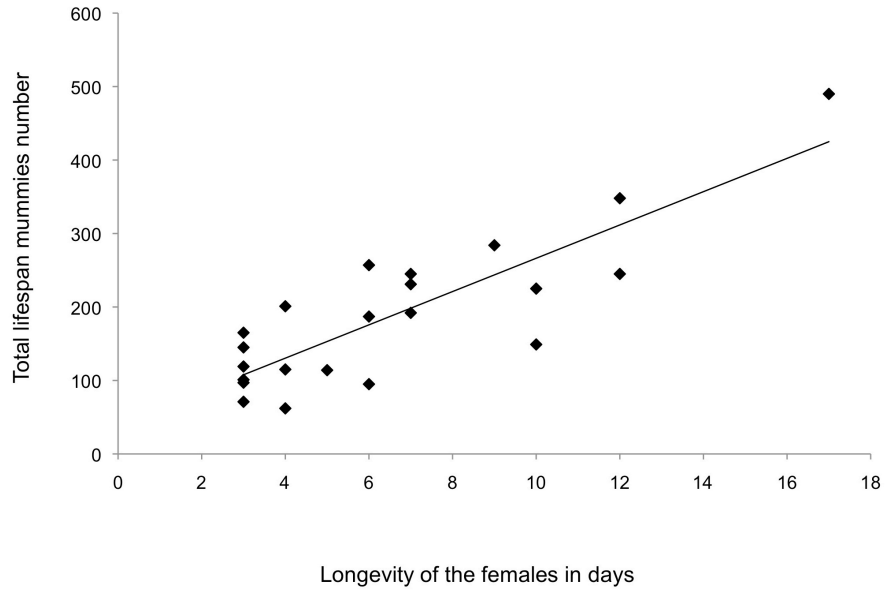

Figure 3. Relation between total numbers of mummies produced by mated females of *A. matricariae and longevity*. N = 22.

According to the AIC criterion, the best models to explain the relation between female age and the probability to produce a male were "female ID + time" and "females ID + life duration" (table 1). The probability to produce a male increases with time in both case, and differ between females ID (table 2).

Table 1. Selection of the best model to explain the relation between female age and the probability to produce a male in *Aphidius matricariae* mated females (n=23). Generalized linear models with a binomial distribution and a logit link function. Models are ranked according to their AIC values. Models significantly differ from each other if ΔAIC > 2.

Parameter	K	AIC	ΔAIC
Time + Female ID	24	4213,42	/
Time + Life duration	3	4336,66	123,23
Time	2	4342,03	128,61
Female	23	4477,28	263,86
Life duration	2	4597,21	383,78
Intercept only	1	4758,74	545,32

Table 2. Best selected model according to their AIC value. The probability to produce a male is positively influenced by time in both cases. In the first model (Day + Female ID), the estimate expresses the difference with the reference (Female 9). In the second model, the probability to produce a male is positively influenced by the Life Duration.

Model time + Female ID				**Model time + Life duration**		
Parameter		**Estimate**	**Std**	**Parameter**	**Estimate**	**Std**
Intercept		-0,591	0,2672	Intercept	-1,2425	0,081
Time		0,2723	0,0181	Time	0,2525	0,0166
	1	0,0923	0,295	Life duration	0,0261	0,0096
	10	-0,1632	0,405			
	11	-0,6009	0,334			
	12	-0,909	0,3092			
	13	-0,38	0,296			
	14	-0,9539	0,3071			
	15	-0,4957	0,2968			
	16	1,4834	0,5361			
	17	-0,7636	0,3563			
	18	-0,8879	0,3719			
	19	-0,3756	0,3384			
Female	2	-1,1221	0,3172			
	20	-1,1706	0,3137			
	21	-0,9906	0,3156			
	22	-0,5142	0,3309			
	23	-0,686	0,322			
	24	-0,6274	0,3153			
	3	0,6215	0,3188			
	4	-0,6549	0,3347			
	5	-0,6314	0,3086			
	6	0,1361	0,3964			
	8	-0,0059	0,3518			
	9	0	0			

When replacing the female ID effect by its life duration, the probability to produce a male increases with life duration (figure 4). However, the model using “time + female ID” better explain the probability of producing male than model using “time + life duration” (delta AIC =123), meaning that the female effect was partly explained by the female life duration.

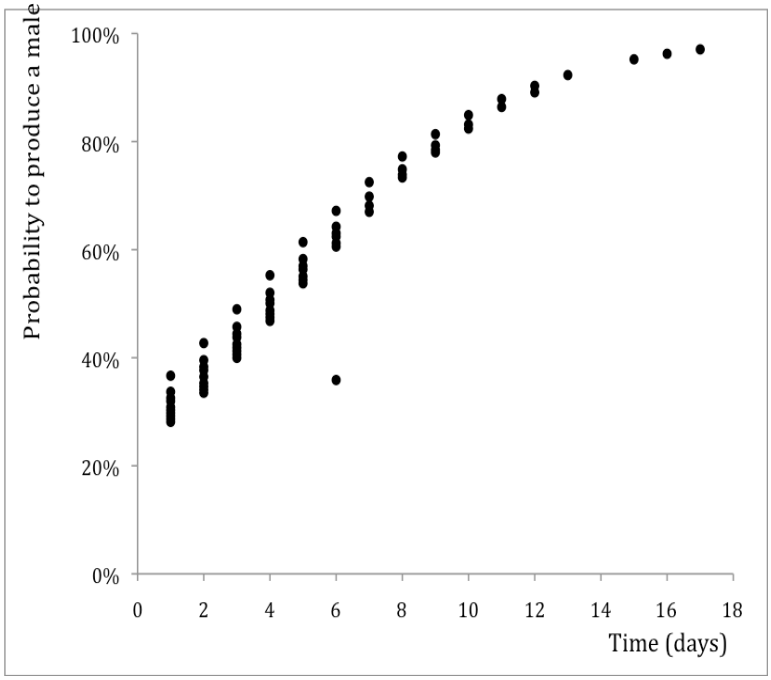

Figure 4. Calculated probability to produce a male according to time, for all females together, n=22.

Independently of their longevity, females produced less mummies (paired t-test, t = 4.22, df = 21, *P* = 0.0004) and more males (paired t-test, t = 4.29, df = 21, *P* = 0.0003) during their last egg-laying period (day of death) than during their first day of life (figure 5).

In all cases after egg-laying, none of the 22 females tested accepted a second mating. The spermatozoid stock of 8 females was thought to be empty because of the production of 100% males during at least their last 24 h oviposition period. The lifespan offspring sex ratio of these 8 females was compared to the offspring sex ratio of the 14 others females (that did not produced 100% of males before death). This comparison did not evidence any statistical influence of the sperm depleted status on the lifespan offspring sex-ratio (t-test, t = 031, p = 0.75).

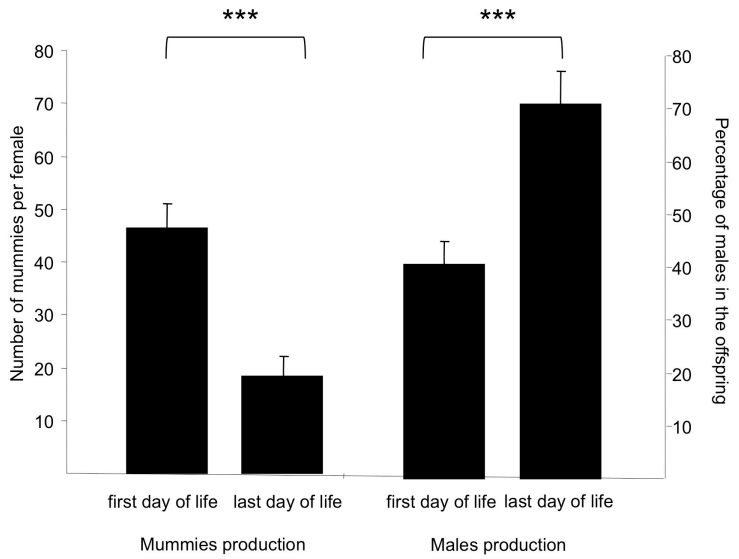

Figure 5. Mummy production (i.e. total number of mummies produced per female, mean ± s.e.) and sex ratio of offspring (mean ± s.e.) produced by females during two different 24h egg-laying periods corresponding to the day of emergence and the day of the death of females. N = 22.

Discussion

Although some studies gave indications that *A. matricariae* is monoandrous (Verai, 1942; Giri *et al*., 1982), our results show that some females can accept mating more than once if they did not have access to hosts. However, egg-laying does not inhibit mating in *A. matricariae* because virgin females that began to lay eggs accept to mate. It represents one of the few reports of mate acceptance of aphid parasitoid females that had begun to lay eggs except the study of Verai (1942) and Suba Rao & Sharma (1962) that concluded opposite results of ours. Moreover, even if the female use sperm for fertilisation of laying eggs, no second mating have been observed once post-mating egg-laying had taken place in *A. matricariae*. As the egg-laying activity of virgin females did not impede mating, the absence of re-mating of already mated females should be due to other parameters.

In our experiment, only females that did not have access to hosts accepted a second mating and the proportion of polyandrous females increased with age and decreased after 6 days old. Males and females prefer

young partners in many insect species (Bondruanski, 2001; McNeil & Brodeur, 1995; Bondruanski et al. 2008; Santiago Anjos Duarte et al. 2011) but not all (Nieberding *et al*., 2012). *A. ervi* females significantly mate less after 5 days old because of their lower attractiveness to the male (McClure *et al.,* 2007; He *et al.* 2004). Similar results were found in *A. matricariae* (Bourdais & Hance, unpublished data), females of more than 6 days mate less with a young male. However, our data shows the evolution of the percentage of polyandrous females with time, whereas other studies on polyandry only compared "young" (usually 1 day old) vs "old" (3 to 10 days old). This is an important result because the proportion of mated females that accept re-mating increases with their age before this crucial age of 6 days old when both virgin (Bourdais & Hance, unpublished data) and mated females became unreceptive to mating. Our results coupled with those on *A. ervi* could support the hypothesis that two opposite effects probably act on the re-mating acceptance of *Aphidus* females: an increasing proportion of mated females that accept re-mating is stopped by the decreasing effect of mate acceptance due to ageing.

Females of many insects can increase their fitness thanks to polyandry and fresh sperm supply (Kraus *et al.,* 2004, Arnqvist & Nilsson, 2000) but this is not observed in *A. matricariae* as supposed sperm depleted females do not re-mate. It is generally assumed that a single mating usually supplies enough sperm to fertilise all eggs in monoandrous insect species (van den Assem, 1986; Arnqvist & Andres, 2006; Collins, 2000). For instance, in *Trichogramma turkestanica*, the 50 sperm stored in the spermatheca are enough for the total number of daughters produced over the female lifetime (Damiens & Boivin, 2005; 2006; Martel *et al*., 2008), but some *Trichogramma* species that live longer may face sperm depletion (King 2000, Leatemia et al. 1995) and polyandry is likely to increase the reproductive success of these females (van den Assem, 1986). The same pattern was observed in other parasitoids under laboratory studies (Hardy & Cook, 1995; Fauvergue *et al.,* 1998; Perez-Lachaud & Hardy, 1999). In field-collected females of *Habrobracon hebetor* 75% of females became sperm depleted at some point before the end of their reproductive lifespan (Ode *et al*., 1997) and less that 5% of them willing to re-mate. We did not count how much spermatozoa are stored in the spermatheca of *A. matricariae* but our results suggest that as for some *Trichogramma* species, the mean amount of stored sperm is enough to be not sperm depleted for the majority of females, but some of them live long enough to suffer from sperm depletion or have different sex allocation strategies (producing more female biaised broods at the beginning of their life). Indeed, the evolution of sex ratio with age in *A. matricariae* is similar of what has been observed in other species. Many parasitoid females produce female biased sex ratio early in life and male biased sex ratios later because of sperm depletion or limited sperm viability (King, 1987; Steiner *et al.,* 2007).

The response towards a second mate between females that did not lay eggs and those that did has never been explored under the same conditions in hymenoptera parasitoids. Our results put in evidence that females behave in different ways whether or not she had access to hosts between two mate proposals and accepted re-mating only without access to hosts. The motivation to re-mate in insect females could either be due to a decrease of sperm number or quality (Thornill & Alcock, 2001). Usually, the spermatheca is able to protect spermatozoa from a decrease in quality (Damiens *et al.,* 2003) but there are some evidences of sperm quality decrease in some species (Cunningham *et al.,* 1971; Tsubaki & Yamagashi, 1991; Reinhardt *et al.,* 1999). A decrease in sperm quality is dependent on sperm age, leading to a decrease in probabilities of fertilisation of eggs (Reinhardt *et al*., 1999). In this study we did not evaluate the quality of the stored sperm over time but some of our results could support this hypothesis. *A. matricariae* laid female biased broods in the beginning of its life and male biased broods at the end. In the absence of intraspecific competition, hymenopteran female parasitoids are supposed to adjust their offspring sex ratio (King, 1987; Mackauer & Völkl, 2002) in order to decrease the mate competition between its sons. In that way, the sex ratio if often female biased (Mackauer & Völkl, 2002; Singh & Pandey, 1997), as seen in our experiments when females are young (1st day of oviposition). Further investigations on sperm quality are now required.

The small percentage of polyandrous females in *A. matricariae* does not mean that the species is polyandrous. Studies on mate number had been done in laboratory conditions that are known to favour polyandry in apparent monoandrous insect species because of the rearing process (Burton-Chellew *et al*., 2007; Mackay *et al.,* 2005). Recently, it has been proved that the longer *N. vitripennis* strains were kept in laboratory, the more frequent polyandrous females became (Burton-Chellew *et al.,* 2007). To our knowledge this study is the first one dealing with this aspect in parasitoids but a similar result was observed in Drosophila (Sgo & Partridge, 2000; Mackay *et al.,* 2005). Our *A. matricariae* strain has been under lab conditions during several years as it is used in mass rearing for commercial purposes. Field studies should now be done to evaluate the proportion of polyandrous females under natural conditions and compare them with our lab strain.

Aphidiinae parasitoid females parasitize colonies of aphids, known to have high variations of population densities between months within a year (Thies *et al*., 2005). Based on this, we can hypothesise that the female parasitoid accepts re-mating only if she had no access to hosts for a long time in order to keep its sperm stock in good condition for future host exploitation. Obviously, the sperm quality as a function of time should be studied to support this hypothesis and to confirm our observations with the evolution of the sex ratio.

Conversely, if the newly mated female has rapid access to aphids, she will begin to lay fertilised eggs that will produce daughters. From this moment, she will refuse any mating probably because the probability of encountering other host patches is presumed to be high (Outreman *et al.,* 2005). In that case, re-mating is not necessary to optimise its fitness because one mate seems sufficient to supply an amount of sperm that will optimise its lifetime sex ratio. Moreover, production of sons at the end of its life due to sperm depletion will still increase the fitness of the female because their sons could also mate and increase its fitness. Further studies coupling host exploitation strategies during the entire life of the females and re-mating should now been done to validate our proposals.

Acknowledgements

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3. Mating window of both sexes: how ageing could affect mating strategies of Aphidius matricariae*?*

Mature mates still sexy in the aphid parasitoid *Aphidius matricariae*

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Abstract

Age can affect insect mating through various ways, from sexual maturation to senescence, and have consequences on both mating strategies and mate choice. Mating is a behaviour that depends on strategies of both sexes that results from intrinsic and extrinsic factors. In this study, we explored the mating window of both sexes of the aphid parasitoid *Aphidius matricariae* (Hymenoptera, Aphidiinae). We analysed how ageing could affect mate choice and acceptance. Males can mate just after emergence, while females need a sexual maturation time of around 30 minutes before being receptive to courting males. Males have a longer mating window than females: whereas more than 20% of more than 8-day-old males are still able to mate with a young female, only 5% of females mate with a young male. Choice experiments showed that young males and females mostly mate with a young partner while old ones do not discriminate. The sex differences in the mating window and mate choice reflect the global life history traits and strategies of *A. matricariae*.

Key Words.

Mate choice, ageing, sexual maturation, mating window

Introduction

The sexual selection theory concept arose from the observation that many animals develop features whose functions are not to help individuals survive, but help them to maximise their reproductive success. Consequently, the choice of mate must lead to the choice of a partner that produces the highest reproductive success and highest quality of offspring (Bateson, 1983). Mate choice has been well studied in a wide range of taxa including insects (Kokko *et al*., 2003; Andersson & Simmons, 2006; Green & Madjidian, 2011). In both sexes, mate choice is the result of extrinsic and/or intrinsic factors that often link physiological or morphological parameters to the observed choice. Individuals maximise their reproductive fitness by reacting to extrinsic signals that indicate the quality of their potential mates (Kirkpatrick & Ryan, 1991; Andrersson & Simmons, 2006). Behavioural displays, morphological ornaments, olfactory signals or any other traits such as individual size, mating status or age advertise mate quality and are used in mate choice in both sexes (Bateson, 1983). During mate choice, individuals must balance the benefits gained by being choosy and the costs of a stronger preference for a potentially better partner. This should result in variation in the expression of mate preferences over the lifetime of an individual. Bateson (1983) suggested that the level of choosiness of a female is a function of its reproductive quality and could change over time. For instance, females of high quality (i.e., virgin, young and big) should only accept mates of a similar high quality (Moore & Moore, 2001). However, individuals with a lower reproductive quality (old individuals for instance) should be less choosy, accepting lower quality males as mates. Gray (1999) demonstrated that older female house crickets showed no significant preference for the calls of attractive males compared with young females. Female cockroaches are also less choosy when old (Moore & Moore, 2001).

Hymenoptera parasitoids are a useful model in behavioural studies concerning mate choice as the mating system of some model species is now quite well-known (Choe & Crespi, 1997; Hardy, 1994; Godfray & Cook, 1997; Jervis, 2005). However, the different components that drive mate choice have not been extensively studied. The size of an individual acts as a positive signal and both males and females prefer to mate with a bigger partner than a smaller one (Bennett & Hoffman, 1998; Bertram *et al.,* 2000; Joyce *et al*., 2009), as size positively influences the fecundity (number of eggs or spermatozoa) of both sexes (Godfray, 1994; Bennett & Hoffman, 1998). If inbreeding has considerable influence on fitness, kin discrimination is selected and males and females prefer to mate with non-kin (Ode *et al.,* 1995), even if some exceptions test the rule (Bourdais & Hance, 2009). Mating status can also reflect mate quality and both sexes more often mate with a virgin partner (Ruther *et al.,* 2007; Allen *et al*., 1994; McNeil &

Brodeur, 1995; Schworer *et al*., 1999; Martel *et al*., 2008a; King *et al*., 2005). Age also acts as a signal for mate choice, individuals usually preferring young partners than older ones (He *et al*., 2004; Martel *et al*., 2008a). The preference for young or virgin partners has often been studied in function of the female age, as sexual pheromones are known to vary with age (McNeil & Brodeur, 1995; Marchand & McNeil, 2000) and the mating status in monoandrous species (McNeil & Brodeur, 1995). For instance, in *Aphidius ervi*, the adult age affect both the emission and the receptivity to sex pheromones produced by the females (McClure *et al*., 2007). Moreover, the effect of a trait (age for instance) should not only be explored on the mate acceptance rate. Other traits play a role during the courtship and could be influenced by age: male wing-fanning behaviour or duration of copulation (McClure *et al*., 2007).

In aphid parasitoids (Hymenoptera, Aphidiinae), studies on the effect of age on mating should be driven by two important aspects linked to the ecological parameters of the species: the mating window (sexual maturation and sexual senescence) and mate choice. Aphid parasitoids are often considered as peudo-gregarious. Indeed, a single parasitoid female may parasitize an aphid colony in a very short time period. Male and female offspring then emerge in nearly the same laps of time, giving them the opportunity to mate before leaving the patch. However, in that case, there is a high probability of sib-mating that should be avoided because of the inbreeding risk, notably for diploid male production (Salin *et al*., 2004). For *Aphidius matricariae,* there is no behavioural avoidance of sib-mating (Bourdais & Hance, 2009). In *Aphidius ervi*, males emerge in average before females but probably not enough to avoid inbreeding (He *et al*., 2004). In that context, the sexual maturation time of both sexes is important to study because it could drive the probability of on-patch mating and inbreeding avoidance. The mating window of aphid parasitoids is almost unknown except one study that showed a sexual maturation time of 4 hours for *A. ervi* males (He *et al*., 2004).

Moreover, males and females have a quite long life expectancy (around 12 days under laboratory conditions, personal observation), meaning that selection pressure favoured a quite long survival. Since finding a mate out of the patch is rare in natural conditions (Fauvergue *et al*., 1999), we predicted that both sexes would be able to mate for a long period of time and that they would accept a quite old partner for mating, even if they were young, as observed in *A. ervi* (McClure *et al*., 2007). On the other hand, given that males are polygynous and females are monoandrous (Giri *et al*., 1982; Verai, 1942), the mating system increases the ecological pressure to find the better mate for the female because she mates only once. In that way, we hypothesised that females should be choosier than males.

Here, the effect of adult age on attraction, courtship capability and mate choice was studied in *Aphidius matricariae* Halliday (Hymenoptera: Braconidae), an aphid parasitoid industrially produced to control various greenhouse aphid species such as *Myzus persicae* (Sulzer) (Boivin *et al*., 2012). In this paper, we report behavioural investigations of male and female mating window by coupling different parameters involved in courtship and copulation such as the time latency and wing-fanning rate, the time latency before copulation, copulation rate and its duration. The consequence of adult age on mate choice in both sexes was tested in choice trials between old and young individuals.

Methods

Rearing of insects

A colony was initiated from individuals originating from Viridaxis S.A. (Belgium), a company that rears and commercialises an Italian strain of *A. matricariae* for the biological control of aphids in greenhouses. In the laboratory, *Aphidius matricariae* was reared on the peach potato aphid *Myzus persicae* (Homoptera: Aphididae) maintained on turnip plants. Aphids and parasitoid cultures were maintained in separate rooms, both at 20±2C°, 70±5% of relative humidity and a photoperiodic regime of 16 hours of light and 8 hours of darkness.

Mated *A. matricariae* females (less than 24-h old) were individually allowed to parasitize a maximum of 100 aphids aged of 3 days placed in a Petri dish for 4 hours. This was done to standardise parasitoid size and avoid any effects of size on mate choice. To obtain naïve individuals for bioassays, all parasitoid pupae (i.e., mummies) were kept individually in 1.5-ml micro-centrifuge tubes until emergence. The mummies were checked at least twice a day and the emergence time recorded. Male and female adults were thus kept virgin and nullipare until the experience.

Mating with one partner

During our experiments, the influence of age on various behavioural aspects of mating was studied on males and females. The “tested partner” is the individual (male or female) that is tested at different ages: 5 min after emergence, 30 min, 1 hour, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days and 12 days. Twelve days was the maximum lifespan of most adults under our laboratory rearing conditions, with full access to water and honeydew (personal observation). The sexual maturation time was detected by choosing small periods of time after emergence (less than 5min, 30 min and 1 hour). For the first period, the

partner was introduced into an Eppendorf® when the tested individual was emerging from the mummy and t=0 was when it had totally emerged.

During the mating experiments, we put one male and one female in a 1.5-ml Eppendorf® and observed them for 5 min. As *A. matricariae* exhibits the same mating behavioural sequence as close relative species (*A. ervi:* Battaglia et al. 2002; He & Wang 2008; *A. rhopalosiphi*: Bourdais et al. 2011), we used different behavioural parameters such as the copulation rate, the copulation duration and wing-fanning to evaluate the influence of age on mating decisions of both sexes (Table 1).

Table 1. Experimental set-up, recorded parameters and their behavioural significance.

Variable age	Fixed age (24-h old)	Observed behaviours	Behavioural significance
Female	Male	Mating rate (% of copulations) Copulation duration (seconds) Time latency before copulation (seconds)	Female recognition of the male and acceptance (Battaglia *et al.* 2002; McClure *et al.* 2007)
		Wing-fanning rate (% of males that express the wing-fanning behaviour) Time latency before male wing-fanning (seconds)	Female attractiveness (Battaglia *et al.* 2002; Steiner *et al.* 2006)
Male	Female	Mating rate (=% of copulations) Copulation duration (seconds)	Male acceptance and capability (Bourdais *et al*. 2012)
		Time latency before copulation (seconds)	Efficiency of male courtship (McClure *et al*. 2007)
		Wing-fanning rate (% of males that express the wing-fanning behaviour) Time latency before wing-fanning (seconds)	Male detection capability of the female (Battaglia *et al.* 2002; Steiner *et al*. 2006; McClure *et al.* 2007)

Mate choice:

To evaluate whether old individuals were more or less selective than younger ones, we designed choice trials. Mate choice experiments were performed in a small glass box named "mate chamber" divided into three zones of 1cm×1cm×1cm each. The tested individual was placed in the central zone and the two potential partners in the left and right zones. At *t*=0, the tested individual was given access (opening of plastic doors) and we noted the chosen mate. Mate choice was recorded for a maximum of 10 minutes. Individuals were directly observed without using any mark. Mate choice was tested by choice tests: one tested partner (young, 1-day-old or old, 7-days-old) had the choice between two partners of the opposite sex that

had the same age (1 and 1 day) or different age (1 and 7 days). Twenty repetitions were performed for all combinations.

Statistical analyses

To clarify the analysis and compare easily the behaviour as a function of the sex, we presented the results of this study according to sex, i.e., first, the behaviours as function of the age of the female, second as function of the age of the male, and then we compared both. The values of the behavioural parameters (mating rate and wing-fanning behaviour) as a function of age were first constant (Y_0) until age X_0 and then underwent a decreasing phase. In this case, the equation was fit to a curve following the formula: $Y = IF(X<X_0, Y_0, Y_\infty+(Y_0\text{-Plateau})*\exp(-K*(X-X_0)))$ (GraphPad Prism). In this formula, X_0 is the age at which the decrease began (or the change in behaviour), Y_0 is the average Y value before age X_0. Y_∞ is the Y value at infinite times and K is the slope of the decreasing phase expressed in $hour^{-1}$ (Figure 1). We analysed individual times (time latency before wing-fanning and time latency before mating) and mating durations as a function of age with one-way ANOVA.

For mate choice trials, $\chi 2$ goodness-of-fit statistics were used to test the mating rate observed when one mate (old or young) had the choice between two mates of the opposite sex (old and young, old or young). The mating ratio was compared to a binomial where both competitors should be chosen with an equal frequency (i.e., expected proportion of 0.5).

Results

Mating with one partner

(a) *The influence of the female age*

The age of the female influenced the mating rate (Figure 1a, Table 2). The values of the mating rate as a function of age are detailed in Table 2. The mating rate stayed constant at the value of 65.3% ($=Y_0$ of the model) until the female was 5.6-days old (135.8 hours, $=X_0$), afterwards a decrease was observed at a rate of 0.080/hours (K) to reach a value of 5.6% when the females were very old ($=Y_\infty$). Thirty minutes after their emergence, most females accepted to mate, while females older than 5 days rarely accepted (Figure 1a).

The wing-fanning rate (Figure 1a, Table 2), an indicator of the detection of a female by a male (Table 1), stayed constant at the value of 81.1% until the female reached 6.4-days old (154.3 hours), afterwards a decrease was observed at a rate of 0.006/hours to reach a value of 0.0% when the females were very old (no detection). Young females increased

more rapidly the wing-fanning behaviour of the males than old ones (one-way ANOVA, f = 10.95, 0.28, p < 0.0001).

The comparison between the mating rate and the wing-fanning rate showed that these two curves had two different plateau values (different values of Y_0, Table 2), but the decrease in detection and mating happened at the same time (similar values of X_0, Table 2). Their values did not decrease at the same rate (different values of K, Table 2), but these two decreasing rates reached the same $Y\infty$ value, meaning that detection and mating reached same values when the females were very old.

There was no effect of the age of the female on the mean time latency before the female accepted to mate (96.81 ± 4.14 sec, one-way ANOVA, f = 1.014, p = 0.43) or the copulation duration (53.12 ± 0.82 sec, n = 225, one-way ANOVA, f = 2, 0.08, p = 0.061).

(b) The influence of the male age

The plateau value of the mating rate stayed constant at 63% until the male was 4.4–days old (106.6 hours), afterwards a decrease was observed at a rate of 0.02/hours to reach a value of 20.60% when the males were very old (Figure 1b, Table 2). It should be noted that in contrast to females, most males were sexually mature just after their emergence from the mummy, with at least 75% of them accepting to mate after 5 min against only 23% of the females. 20% of males that were older than 6 days accepted to mate in our laboratory conditions.

The wing-fanning rate of the male (Fig. 1b, Table 2) stayed constant at the value of 90% until the male was 3.9 days (94.2 hours), afterwards a decrease was observed at a rate of 0.004/hours to reach a value of 0.00% when the males were old (no detection of the young female).

The comparison between the mating rate and the wing-fanning rate showed that these two curves were totally different (Table 2).

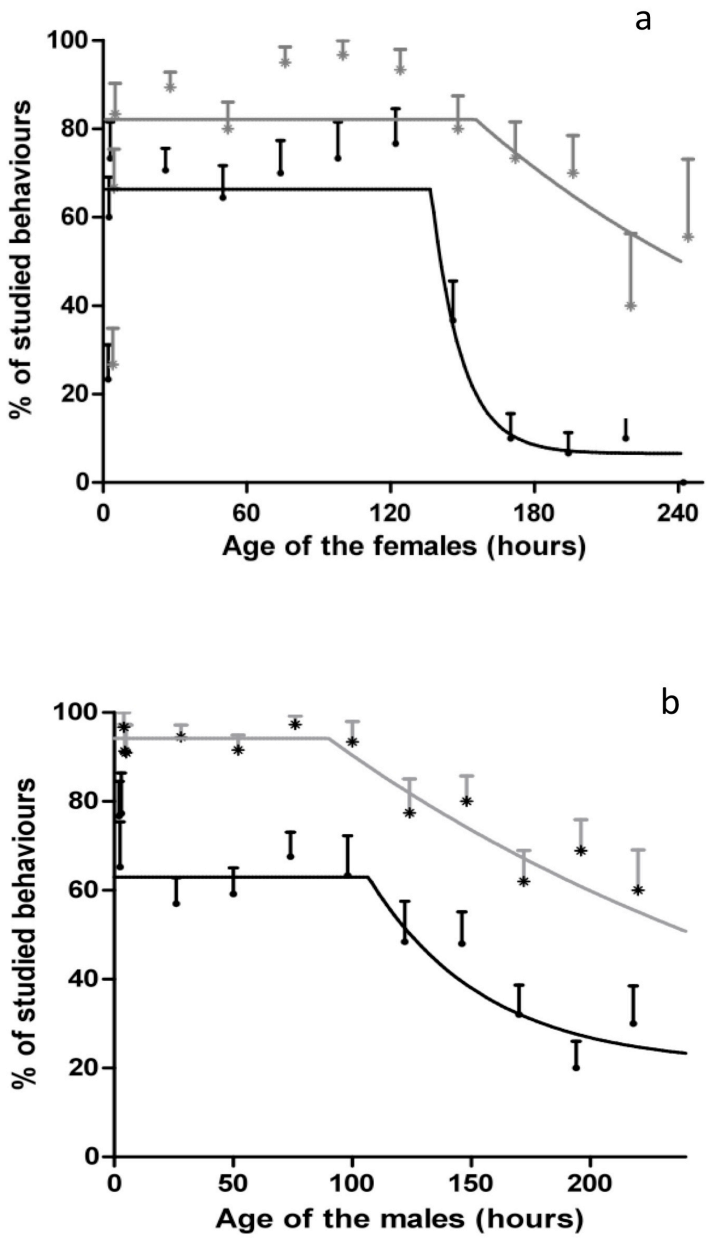

Figure 1. Changes in mating rate (black lines) and wing-fanning rate (grey lines) with the age (in hours) of the female (a) or the male (b) of *Aphidius matricariae*. Data are given in %±IC.

Table 2. Mating and wing-fanning rates as a function of male and female age. Comparisons were significantly different when put in bold. X_0 is the time (hours ± s.e.) at witch the decrease begin, Y_0 the average percentage of expression of the behaviour (± s.e.) before age X_0, $Y\infty$ is the Y value (% of behaviour ± s.e.) at infinite times, K is the slope of the decreasing phase (jour^{-1} ± s.e.).

Parameters as a function of the…		Mating rate		Wing-fanning rate	
		♀ age	♂ age	♀ age	♂ age
Best-fit values	X_0	135.8 ± 3.4	106.6 ± 5.26	154.3 ± 14.99	89.97 ± 2.84
	Y_0	65.31 ± 0.8	63.0 ± 0.86	81.14 ± 0.99	94.17 ± 0.41
	$Y\infty$	5.6 ± 2.5	20.58 ± 3.24	0.00 ± 195.6	0.00 ± 20.65
	K	0.08 ± 0.03	0.02 ± 0.005	0.006 ± 0.02	0.004 ± 0.001
Goodness of fit	R	0.85	0.88	0.36	0.91
	df	425	266	425	554
F tests comparison between gender P, F value (DFn;DFd)		**Mating rate**		**Wing-fanning rate**	
	X_0	0.40, 0.71 (1;691)		**< 0.0001, 37.87 (1;979)**	
	Y_0	0.09, 2.90 (1;691)		**< 0.0001, 151.8 (1;979)**	
	$Y\infty$	**0.03, 4.74 (1;691)**		0.92, 1.01 (1;979)	
	K	**< 0.001, 10.92 (1;691)**		0.11, 2.58 (1;979)	
F test comparison between behaviour, same gender P, F value (DFn, DFd)		Comparison mating rate *vs.*fanning rate ♀			
	X_0	0.16, 1.96 (1;850)			
	Y_0	**< 0.0001, 158.4 (1;850)**			
	$Y\infty$	0.96, 0.003 (1;850)			
	K	**< 0.0001, 40.63 (1;850)**			
		Comparison mating rate *vs.*wing-fanning rate ♂			
	X_0	**0.005, 7.82 (1;820)**			
	Y_0	**< 0.0001, 460.8 (1; 820)**			
	$Y\infty$	**0.02, 5.25 (1; 820)**			
	K	**< 0.0001, 31.02 (1; 820)**			

When the wing fanning behaviour began, all males that had expressed this behaviour expressed it after an equal latency (43.59 ± 2.59 sec, n = 488, one-way ANOVA, f = 1.73, 0.07, p = 0.053). Mating lasted 55.03 ± 0.92 sec (n = 317) and we did not find any effect of male age on their duration (one-way ANOVA: f = 1.57, 0.06, p = 0.091). There was also no influence of the age of the male on the percentage of males that recognised (wing fanning) young virgin females but failed to mate ($\chi 2$ = 15.58, p = 0.33).

(c) Comparison of the two sexes:

The comparison between the dynamics of the mating rates of the two sexes showed that these two curves had two similar plateau values (similar values of Y_0, Table 2) and the decrease in mating happened at the same age for both sexes (similar values of X_0, Table 2). Their values did not decrease at the same rate, the mating rate of the females decreasing more deeply than that of the males. The two decreasing rates reached different $Y\infty$. 20% of the

very old males still mated while, at the same age, only 5.6% of females mated. The comparison between the dynamics of wing fanning of the two sexes showed that these two curves had two different plateau values (Table 2) and the decrease in mating happened at different ages. The curves of the wing-fanning rate as function of the age of the mates had two similar parameters, K and $Y\infty$ (Table 2). When the influence of the female's age was tested, it was observed that the wing-fanning rate was higher and stayed constant for a shorter period than when the male's age was tested.

*Mate choice***:**

(a) The influence of the female age

The mating rate of young and old females was the same regardless of being in contact with 2 old or 2 young males (Table 3). In the choice tests, when old females had the choice between 2 males, one young and one old, the mating rates were not statistically different (Table 3), but young females more often mated with the young male (Table 3). When a female was presented to 2 males, we did not find any influence of the female age on the time elapsed before copulation in any of the choice trials (Krustal-Wallis test, k_6=6.369, p = 0.27).

(b) The influence of the male age

The mating rate of young and old males was the same regardless of being in contact with 2 old or 2 young females (Table 3). In the choice tests, when old males had the choice between 2 females, one young and one old, the mating rates were not statistically different (Table 3), but young males mostly mated with the young female (Table 3). There was an influence of the male age on the mean latency time before the copulation occurred (Krustal-Wallis test, K_6 = 17.83, p = 0.0032). Dunn's multiple comparison tests revealed that old males spent less time before copulation when presented with 2 young females (41.12 ± 3.14 sec) than young males (77.5 ± 13.12 sec) (Dunn's post-test, $p<0.05$). Old males that had to choose between old or young females spent 88.67 ± 13.43 sec before copulating, which was significantly longer than when they had to choose between 2 young females (41.12 ± 3.14, Dunn's post-test, $p<0.01$), but the same as when they had to choose between 2 old females (85.50 ± 15.58, Dunn's post-test, $p>0.5$).

(c) Comparison of the two sexes

Both sexes behaved in the same way when choosing between two partners of the same age (Table 3). Mate preferences were also the same. Young individuals of both sexes preferred young partners when they had the

opportunity to choose, but old males and females mated equally with old or young partners if both were present (Table 3).

Table 3. Male and female mating rates in choice tests as a function of age. Fisher exact tests were used and p values are given in the table.

		Age and sex of the tested parasitoid					
		Females			Males		
		young (1 day)	old (7 days)	Comparison young *vs.* old	young (1 day)	old (7 days)	Comparison young *vs.* old
Mate acceptance							
Age of the parasitoid partner	2 young partners	85%	55%	p=0.08	90%	90%	p=1
	2 old partners	90%	75%	p=0.40	85%	75%	p=0.69
Comparisons between young and old partners		p=0.63	p=0.32		P=1	P=0.40	
Mate preference							
Preference in choice tests between old and young		**young P=0.01**	no choice P=0.49		**young p=0.02**	no choice p=1	

Discussion

Our results demonstrated a gender difference in the mating window of the parasitoid *Aphidius matricariae*. For both sexes, the mating rate followed a curve with a plateau phase followed by a decay phase. During the first six days, the mating rate was not influenced either by the male or female age. Afterwards, the mating rates decreased dramatically, but competition during choice trials masked this tendency because old individuals had a higher mating rate. Mating rates did not decrease at the same rate; the mating rate as a function of female age decreased more deeply than that of males. Twenty per cent of the very old males (10 days) still mated, while at the same age, only 5.6% of females mated when presented with a partner.

Most females needed around 30 minutes after emergence to accept to mate, while most males were sexually mature just after their emergence. A period of sexual maturation for either males or females has been reported in various insect species (Thornill & Alcock, 2001) and in parasitoids ones (Charnov, 1990; Quimio & Walter, 2000; Drea *et al.,* 1972). In quasi-gregarious species (i.e., if hosts of a solitary species are gregarious, such as aphids), there is, respectively, no and partial local mating and variable period of sexual maturation among species. *Pachycrepoideus vindemiae* is a quasi-gregarious parasitoid of pupae where males emerge a day before females and

wait on the natal patch to mate with newly emerged females (Nadel & Luck, 1985). *Fopius arisanus*, a tephritid fruit fly parasitoid, has a male sexual maturation time of 4 days, the necessary time for the migration of sperm from the testes to the seminal vesicles (Quimio & Walter, 2000). The sexual maturation time in aphid parasitoids is mostly unknown. The study of He et al. (2004) on *Aphidius ervi* showed that newly emerged females were able to entice the courtship display but 12-hour-old females mated more. Newly emerged males were able to perform courtship, but failed to mate until they were 4 hours old. We obtained different results for *A. matricariae*. Females were reluctant to mate for 30 minutes after emergence, but the males did not need a sexual maturation time as seen in *A. ervi*. Thus, contrary to *A. ervi*, on-patch mating seems possible in *A. matricariae* if males and females wait for enough time on the patch before dispersing. On the other hand, the short period of being unreceptive (30 minutes for females) could be sufficient to avoid sib-mating if many siblings emerge at the same time on a patch as they do not have behavioural mechanisms to avoid sib-mating (Bourdais & Hance, 2009). We thus need data about the post-emergence behaviour of *A. matricariae* to understand if mating on the patch is possible or not. If yes, females should stay on their natal patch for more than 30 minutes to mate.

Ageing of both sexes affected mating, but had fewer consequences on males than on females. In our experiments, few old females (more than 6 days old) induced young male recognition because we observed a decreasing proportion of males that displayed wing-fanning behaviour with increasing female age. Indeed, even if some young males exhibited wing fanning, indicating that they recognised the old female as a potential partner, few of them could mate efficiently. This is confirmed by mate choice experiments where we observed that young males preferred to mate with young females when they had the choice. This tendency has already been observed in closely related species where females aged from more than 6 days attract fewer young males (He *et al*., 2004; McNeil *et al*., 2007). Two non-exclusive hypotheses could explain these results. First, female age affects sex pheromone production, especially short-distance ones that stimulate male courtship (Battaglia *et al*., 2002; Schworer *et al.,* 1999; McClure *et al.,* 2007; McNeil & Brodeur, 1995). This could explain the lower attractiveness of young males toward an old female in *A. matricariae*, even if chemical exploration (determination of the nature of the sexual pheromone and it kinetics) is still needed to improve our observations. Second, a study in *Aphidius ervi* (Isidoro *et al*., 1996) showed that the antennal glands in males produced a secretion that acted as a contact pheromone, which elicited mating in males, and that the emission of its secretion was stimulated by the presence of a female. In the no-choice experiments, some males tried to mount old females but no mating occurred, which favours this hypothesis. However, our choice trials did not confirm this because old females mated more easily when they were in competition for one male.

Very old males recognised young females less because few of them displayed wing fanning when aged more than 8 days. However, the changes in the responsiveness of *A. matricariae* males were less pronounced than in other parasitoid species (Damiens & Boivin, 2005; Martel *et al*., 2008b), even closely related ones (McClure *et al.,* 2007; He, 2008). We observed that most old males (7 days old) were still able to mate with a young female both in experiments with one or two partners. Old males remained sexually attractive to females for longer than old females, suggesting that ageing affected males less than females. Ageing could affect male fitness via the number and quality of the sperm they can transfer to the female during copulation (Jones & Elgar, 2004; Jones *et al*., 2007; Pizzari *et al*., 2008). Little data exist on male sperm production in parasitoids (Boivin *et al*., 2005), and even less in aphid parasitoids (He, 2008). The only study conducted in aphid parasitoids suggested that males are moderately syn-spermatogenic because they can replenish sperm if allowed a 24-h period between two mates (He, 2008). This study also showed that old males were still able to inseminate females with a sufficient amount of sperm (He, 2008). If *A. matricariae* share the same spermatogenic index as *A. ervi*, this could explain why old males are still able to mate and court females.

The level of choosiness can fluctuate among individuals and between sexes, reflecting strategic allocation of mating resources (Engqvist & Sauer, 2000). When the relative mating effort is low, i.e., when the cost of the current mating or the probability to find a partner is low in comparison to the future one, the individual is less discriminating (Barry & Kokko, 2010). Moreover, recent theoretical and empirical studies (Kokko & Ots, 2006; Werner & Lotem, 2006; Uetz &, Norton 2007) have shown that mate choice follows different rules depending on whether potential mates are encountered simultaneously or sequentially. This could explain why old individuals acquire more mates when under direct competition. However, mate choice related to age under a competition context has been poorly studied and unexplored in parasitoid species, despite its potential importance in the understanding of the mating strategies of the species. For monoandrous females, there is a trade-off between rejecting a potential mate, even if it is of bad quality, and staying virgin for a long time. Even if hymenopteran parasitoid females increase their fitness by producing sons when virgin or sperm-depleted, they gain more by being mated and producing daughters (Godfray, 1994). Since *A. matricariae* females are monoandrous (Giri *et al*., 1982), their level of choosiness decreases with age. Accepting to mate, even with an old male, is better for them than staying unmated all their life. This could explain why old virgin females accept to mate with old males under laboratory conditions. Males typically have a steeper Bateman gradient (regression of reproductive success against mating success) than females (Barry & Kokko, 2010). Being choosy tends to reduce the reproductive success of a male more than it does for a female (Barry & Kokko, 2010), thus possibly explaining why old males are not choosy and

mate with old or young females. Young males obtain more mates in the no-choice experiments than old ones, probably because they respond more easily to the female presence (wing fanning behaviour) than older males. However, even if young females more often choose young males, old males remain equally able to mate with old than young females. Our data suggest that young males are more able to obtain mates than old ones, but the latter are still able to be as competitive as young males toward a young female in direct competition.

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4. Mate choice and sib recognition

Lack of behavioural evidence for kin avoidance in mate choice in a hymenopteran parasitoid (Hymenoptera: Braconidae).

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Abstract

Mechanisms for inbreeding avoidance should be prevalent in insects that reproduce by arrhenotokous haplodiploidy because of the higher potential production of unviable diploid males in inbred mating. Few studies have focused on mating strategies in insect parasitoids and even less on kinship relationships during mate choice. In this study, we tested avoidance of kin as mate in the parasitic wasp *Aphidius matricariae* (Hymenoptera: Braconidae) using an ethological approach. Key mating parameters, such as male wing fanning, latent period before genitalia contact and duration of copulation were measured. No evidence for kin avoidance in mate choice in both *A. matricariae* males and females was observed in our behaviour (no choice or choice tests) tests. This lack of ethological sibmating avoidance could be due to different factors such as a sex determination rule different than the single locus Complementary Sex Determination, making lower the proportion of diploid males in case of sibmatings and thus its negative consequence. The existence of other inbreeding avoidance strategies and mechanisms that reduce the probability of 2 receptive relatives meeting in nature may be common, for example, inbred mating may be rare through differential dispersal, delayed maturation, or protandry.

Key words: *Aphidius matricariae*, mating strategy, sibmating, inbreeding, kin recognition

Introduction

Kin recognition – i.e. the ability to identify and respond differentially to genetically related individuals – is one of the fastest growing and most exiting areas of behavioural ecology (Ode *et al*., 1995; Pusey & Wolf, 1996; Blouin & Blouin, 1988; Lize *et al*., 2006; Martel *et al*., 2008). Models suggest that sibmating is unlikely to evolve in animals where inbreeding depression and male mating cost are high (Bulmer, 1973; Greenwood *et al.,* 1978; Getz *et al*., 1992).

Consequences of inbreeding depression in a determinate species can be explored in the light of sex determination mechanisms. Haplodiploid organisms may be less prone to inbreeding depression than diploid ones because of lower deleterious recessive allele frequencies (Brückner, 1978; Werren, 1993). Deleterious recessive alleles are generally exposed to selection when they occur in males, the haploid sex. However, inbreeding depression may be expressed in diploid females for some traits such as fecundity (King & King, 1995). Depending on the sex determination model of a particular species (single locus complementary sex determination - slCSD - or multiloci complementary sex determination - mlCSD, see Cook & Crozier, 1995 for more details), the incidence of homozygosity is always higher under inbreeding than under outbreeding and consequently results in increased diploid male production (Cook & Crozier, 1995). In nearly all cases studied so far in hymenopteran species, diploid males are a reproductive dead end because of their low viability (Petters & Mettus, 1980), sterility (Cook, 1993; Stouthamer *et al.,* 1992) or because of the production of diploid sperm and thus sterile triploid offspring (Agoze *et al*., 1994). Thus, selection for mechanisms that limit sib mating should be greater in haplodiploid species that reproduce by slCSD than in species with mlCSD. Inadequate mate choice can be costly through the rejection of good mates and the acceptance of bad mates (Parker, 1983). Costs are sex dependant in a species where males are polygynous and females are monoandrous. For males, costs dependent not only on the number of females they inseminate but also on energy allowed in sperm production (Boivin *et al*., 2005). In cases of a slCSD strategy, mating with a brother will be very costly for monoandrous females if half of the diploid offspring develops into sterile males. Hence, in both cases of sex determination strategies, monoandrous females are predicted to be more selective in choosing their mate than polygynous males. To test this hypothesis we used *Aphidius matricariae*, a hymenoptera aphid parasitoid frequently used for commercial biological control, known to be polygynous and monoandrous (Giri *et al*., 1982; pers. obs.). Its behavioural mating sequence is similar to that of other related species (*Aphidius ervi*, Battaglia *et al.,* 2002, *Aphidius picipes*,

Amice *et al*., 2008; *Aphidius rhopalosiphi*, pers. obs.). No data are presently available on the sex determination strategy in *A. matricariae*.

We thus predict that females are choosier than males in *A. matricariae* and will have developed mechanisms for inbreeding avoidance. Here, we test for kin avoidance by quantifying male and female courtship and mating behaviours in experiments with or without mate choice.

Material & Methods

- *Study organism and rearing :*

Aphidius matricariae (Hymenoptera: Braconidae) is a solitary parasitoid wasp attacking a wide range of aphid species. In the laboratory, 2 different populations of *A. matricariae* were separately reared on peach potato aphids *Myzus persicae* (Hemiptera: Aphididae), which were raised on turnip plants (*Brassica rapa* L. subsp. *rapa*). Aphid and parasitoid cultures were maintained in separate rooms under identical standardized conditions (Temperature: 20±2C°, Relative humidity: 70±5% and Photoperiod: L16:D8).

To obtain different female lines, several mated females of each population individually parasited a maximum of 100 aphids aged 2 days during 4h in a Petri dish. Every parasitoid pupa (i.e. mummies) from each female line was individually kept in 1.5 ml microcentrifuge tubes until emergence. The mummies were checked twice a day and only freshly emerged adults were used for experiments.

- *Experiment 1. Male behaviour and female acceptance*

Through courtship and mating behaviours observations, male behaviour and female acceptance were simultaneously tested. One naive, virgin male was placed in a clean 1.5 ml microcentrifuge tube with one naïve, virgin female for no longer than 5 minutes. In half of the experiments (33 repetitions), males and females were sibs, while in the other half (31 repetitions) pairs were unrelated (i.e. male and female originated respectively from populations 1 and 2). The following behaviours of the mating sequence were quantified: (1) the latency time before wing fanning commenced, which is related to the excitation of the male and to his perception of a receptive female (other experiments have shown that this behaviour does not happen with a mated unreceptive female), (2) the time before genitalia contact which should reflect female acceptance (Battaglia *et al*. 2002), (3) the time of copulation (i.e. time of genitalia contact). All

durations were for wasps that did choose a mate within 5 minutes. The percentage of mating in both treatments was also quantified.

- Experiment 2. Male and female preference in choice tests

Mate preference was tested by choice tests between related and non-related partners of the opposite sex. This experiment was performed in an experimental chamber divided into three zones of 1cm x 1cm x 1cm each. The tested individual was placed in the central zone and the two potential partners in the left and right zones. At t=0, the test individual was given access (opening of doors) and mate choice was recorder during a maximum of 10 min. We tested the following combinations: male choice for a sister or a non-relative (n=36), female choice for a brother or a non-relative (n=37) and 4 control situations (male *vs.* 2 sisters, n=34; male *vs.* 2 non relatives, n=29; female *vs.* 2 brothers, n=15; females *vs.* 2 non relatives, n=25).

Results

- Experiment 1. Male behaviour and female acceptance

Females accepted to mate with a brother as readily as with a non relative male (brother: 80%, non relative: 82%, X_2=0.094, df=1, p=0.759). Females accepted a non relative as mate as soon as a brother (88.9 ± 9.6 s, n=29 versus 96.9 ± 11 sec, n=28; t=-0.57, df = 51, p=0.569). Males fanned to non-relative females as soon as to sisters (37 ± 8.9 s, n = 35 versus 30.4 +/- 6.7 s, n = 33; t = 0.60, df = 62, p = 0.55). There was no significant effect of the degree of kinship on the duration of the copulation (non relatives: 55.9 ± 1.7 sec, n=29 versus relatives: 57.5 ± 2.3 sec, n=28; t=-0.58, df = 51, p=0.566).

- Experiment 2. Male and female preference in choice tests

No differences were observed in the percentage of mating when a female was put in the mating chamber with 2 potential partners (respectively 2 non relatives, 2 brothers, 1 non relative and 1 brother) (respectively 73.33%, 80% and 86.11%, X2=1.21, p=0.545). Similar results were observed for males (respectively 91.17%, 75.86% and 70.27%, X2=4.91, p=0.08). Males and females equally mated with respectively a sib or a non relative in choice tests (males: 42.3% *vs.* 57.7%, X2=0.310, p=0.057; females: 41.93% *vs.* 58.07%, X2=0.399, p=0.53).

Discussion

In the present study, our results demonstrate no behavioural evidence for avoidance of kin as mates by both males and females of

Aphidius matricariae. Other mechanisms that decrease the probability of sib mating in nature may exist. For example, sibmating could be reduced by males developing earlier than females (i.e. protandry) and by dispersal of both sexes soon after emergence (Thornhill & Alcock, 2001). The latter is partly dependent on whether the parasitoid is gregarious, solitary or quasi gregarious. Gregarious parasitoids favour sibmating on the natal patch and local mate competition (Mackauer & Völkl, 2002). Some studies in *Trichogramma* sp. have shown that protandrous males disperse from their natal patch after mating and that a weak proportion of dispersing females are still virgin (Martel & Boivin, 2004). Solitary parasitoid species favour dispersal from natal patch and outbreeding (Nadel & Luck, 1992). If hosts of a solitary parasitoid species are gregarious, e.g. aphids, quasi-gregarious brood can be produced, where partial sibmating and local mate competition can occur (Mackauer & Völkl, 2002). In *Aphidius* sp., males tend to emerge before females (He *et al*., 2004; pers. obs.) but no studies have focused on post emergence behaviour and dispersive behaviour of males and females. However, sib mating could occur in *A. matricariae* in some particular cases such as in the beginning of spring when aphid populations are low.

Moreover, deleterious consequences of sibmating in this species should be compensated by a sex determination mechanism such as ml-CSD. So far sl-CSD has been identified in more than 40 species within four superfamilies of Hymenoptera: sawflies (Tenthredinoidea), parasitoid wasps (Ichneumonoidea), ants and wasps (Vespoidea), bees (Apoidea) (Stouthamer *et al*., 1992; Cook, 1993; Periquet *et al*., 1993; Cook and Crozier, 1995; Heimpel *et al*., 1999; Butcher *et al*., 2000; Beukeboom, 2001; Zayed & Packer, 2001). No data are presently available on the sex determination rule of *A. matricariae*. However, Salin *et al.* (2004) suggested that *Aphidius rhopalosiphi* de Stephani-Peres reproduces under mlCDS and recently slCSD was shown to absent in some other braconid wasps (Beukeboom *et al.,* 2000; Niyibigira *et al*., 2004). Moreover, CSD has been ruled out in other hymenopteran species belong to the superfamily Chalcidoidea in which sib mating is prevalent and in which prolonged inbreeding does not lead to diploid male production (Fabritius, 1984; Legner, 1979; Schmieder and Whiting, 1947; Skinner and Werren, 1980).

Further studies on the consequences of sibmating (i.e. proportions of diploid males, fitness of females) should be conducted enable a better understanding of mating system and sex determination mechanism in this species.

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5. Discussion

The experiments conducted in this chapter confirm that *A. matricariae* has life history traits more similar to a solitary species than quasi-gregarious one and that some mechanisms decreasing the inbreeding probabilities have been selected.

Sex ratio and Local Mate Competition

We did not explore in details the **sex ratio** variations in *A. matricariae* but in our rearings we observed that the sex ratio is near 0.4 (40% of males), meaning a lightly female-biased sex ratio. This was confirmed by the results obtained during the experiment about monoandry when we followed the oviposition of females during all their life. It appears that females lay eggs with a female biased sex ratio if they are alone in the Petri-dish but this may change between days, although the quality of the host is stable. If some various selected behaviours lead to a low proportion of inbreeding in natural populations, females do not manipulate so much the sex ratio of their brood (Fauvergue *et al.,* 1999), this is probably the case in *A. matricariae*. Additional data and experiments are now important to conduct to better understand how *A. matricariae* females adjust the sex ratio of their broods and the relation with the degree of Local Mate Competition.

Use of pheromones?

In the context of Local Mate Competition, rapid male dispersal from their natal patch and attraction towards virgin females promote off-patch mating. If males detect virgin females from a distance, they may disperse to other emergence sites where they can compete with local males (Nunney & Luck, 1988). Indeed, one of the key elements in partial LMC is off-patch attraction. However, **sex volatile pheromones** used for long distance mate attraction are widespread in solitary parasitoids but also occur in gregarious or quasi-gregarious ones (Fauvergue *et al.,* 1999). In close relative species of *A. matricariae*, field and olfactometer methods had put in evidence the role of volatile sex pheromones (*A. colemani*: Reed *et al.,* 1994; *A. nigripes*: McNeil & Brodeur, 1995; *A. rhopalosiphi*: Decker *et al.,* 1993; Bourdais *et al.,* 2012). It is reasonable to think that the use of sexual pheromones is also the case in *A. matricariae* but they are not presently identified and had to be tested in wind tunnels or field conditions.

However, we conducted some behavioural experiments to understand the olfactory responses of males and females towards various **kairomonal odours** but results were not exploitable for further reasons.

First, it appears that *A. matricariae* does not respond in Y-tube olfactometer as other close relative species do. In the laboratory, some experiments in the Y-tube had been done with *A. ervi*, *A. rhopalosiphi* and *A. matricariae*. It appears that our species do not "respond" in the tube meaning that it does not make a choice and move in the tube. In our experiments, less than 50% of females and 30% of males made choices towards attractive odours and this was confirmed by others studies (Mailleux 2011). *A. matricariae* should respond to odours as others species do but further investigations are still missing. Maybe it does not respond because it is too sensitive (or not) to the experimental design and the air-flow in the y-tube, or maybe it needs to fly to orientate itself towards an odour.

Monoandrous females that sometimes succumb to male attempts

The **monoandry** of the parasitoid females is often cited in papers but also often untested. Ridley in 1993 cited that "*most of the source papers were written for purposes other than describing mating frequencies and therefore often do not reveal their evidence. Many authors just write things like 'the female is typically monoandrous" (...) without indicating the observation conditions, sample size or opportunities for remating*". It is quite easy to prove that a female is polyandrous but less to prove that it is monoandrous. However, it appears that solitary species are more often monoandrous and gregarious species polyandrous (Ridley, 1993).

We found that *A. matricariae* female can accept a second mate in some rare and specific situations. It is interesting to note it for a theoretical point of view but the conditions required for polyandry seems difficult to obtain in a natural population because female should not lay eggs between 2 mates. A recent study of He & Wang (2008) on *A. ervi* suggested the same results. The proportion of remated females is near 20% in the laboratory conditions with a high male-biased sex ratio. They also found that the mate duration was shorter in the second mating than in the first one. In 2007, McClure *et al.* also observed that 25% of *A.ervi* females remated 24h after the first one. They supposed that remating is possibly the result of an unsuccessful first mating or due to a low quality mate. Studies that observe the mating numbers of females in aphid parasitoids are still not common but they all suggest that *Aphidius* females are not as strictly monoandrous as we previously thought. This suggests that sperm competition could happen, making sexual selection towards males higher. However, no information is available on the explanation of remating motivation in A*phidius sp.* probably because the levels of remating are low and difficult to obtain for more precise investigations. Nevertheless, it should be interesting to understand why some of them accept to remate to better understand the global mating strategies of the species.

Not a very choosy species… but probably enough to optimize their fitness

Males and females have a quite large **mating window** in laboratory conditions. If natural conditions allow the same window, it is advantageous because it ensures that females can get matted for a long time after emergence and that males can stay competitive for a long time.

Males can mate just after emergence witch favour a mating on the patch of emergence if there are receptive virgin females. This is different from the results obtained in a close relative species, *A. ervi*, where males need 4 hours to be capable of courtship and mating (He & Wang, 2008). However, an aphid parasitoid male seems to be capable of mating even if old (McClure *et al.,* 2007; He & Wang, 2008; this study, paper 4). Their large mating window is important because it potentially allowed more copulation during their life that could help them to maximize their fitness. We do not know if *Aphidus* males are prospermatogenic or not (Boivin *et al.,* 2005), which is very important if we want to better understand their sperm allocation strategies. Some explorative experiments made on this strain showed that males can accept to mate with 4 to 8 females within 4 hours but they all need a minimum of 15 minutes between 2 mates (n = 10, Bourdais, unpublished data). However, we do not have any data on whether they could be sperm depleted or not in that case of rapid matings. Of course, further studies are essential now to understand if and/or how they discriminate and adjust the amount of sperm they give to each female.

Concerning females, it seems that something change in their physiology after day 6 after emergence. Moreover, 61% (n = 23) of the females do not live long enough in lab conditions to reach this age when they lay eggs every day. We thus think that under natural conditions females probably not live more than 6-7 days because of the energy allowed in oviposition and searching for hosts, but also because of the risk of predation. Maybe they are pro-ovogenic and the eggs can not been maintained in good conditions during more than 6 days. We observed a diminution of the number of mummies produced after 6 days old in the 9 females that had lived more than this age in lab conditions. That is probably two reasons why such a mating window was selected in this species. Nerveless, we need more data about the egg production of the species and data of survival under more natural conditions. Females also accept to mate even if they had begun to lay eggs (males only) in aphids. This is very interesting because it invalidate the results of Verai (1942) but it better fits with the ecological characteristics of the species. As for males, further studies on the mating strategies linked to oviposition strategies of the females have now to be done.

Aphidius matricariae males and females seem to **not be very choosy** when it comes to mating. They accept to mate with old partners (except if they have to choose between one young and one old) and mate with their brother or sister indifferently.

Mating with old partners could present disadvantages for both sexes because old males could transmit deleterious spermatozoids and old females could not have a good fecundity. For both sexes of *A. ervi*, younger individuals (1 day old) have higher mating success than older (5 days old) (He & Wang, 2008). In this species, the percentage of daughters produced by old males is significantly lower than for young males (He & Wang, 2008). It could be due either to male sperm depletion either to diminishing sperm viability and mobility related to ageing (Srivastava & Singh, 1995). We also know that females sex pheromone production and release decrease with females' age (Schwoler *et al*., 1999; 2005; Battaglia *et al.,* 2002), which could make older females less attractive for males. In the same time, older males may have decreased ability to recognize and/or respond to female sex pheromones and court or mount females (Cheng *et al.,* 2003; Damiens & Boivin, 2005). In *A. Matricariae,* we did not find any effect of ageing on the mating success of males aged of less than 8 days whereas females more than 6 days old showed a lower propensity to accept mates. However, mesurement of the fitness should be done to valitate our observations.

Sib mating has not been explored in aphid parasitoids. This is probably because we thought that aphid parasitoids are quasi-gregarious with a quite high level of LMC and most of mating occurring on the emergence patch. By doing so, mating between brother and sister could happen and had thus never been tested. Mating between sibs should had been unselected if the consequences are high in term of fitness for one or the other sex. A explorative study on the consequences of sib-mating on this species over 4 generations had concluded that *A. matricariae* does not seems to suffer from inbreeding in terms of fecundity of the females and sex-ratio (Brasseur, 2010) after the first generation only. This study confirms our results: no behavioural difference appears in the different behavioural patterns during mating and sib mating has no effect on the sex ratio, emergence rate. However, it seems that modifications in the sex ratio appear after 3 generations either for *A. matricariae* and *A. erv*i (Brasseur, 2010). Salin *et al.* (2004) founded the same results on *A. rhopalosiphi* and suggested that the species is under sl-CSD. Further investigations are still needed in *Aphidius* species to understand sex determination rules and consequences of inbreeding. Comparison between close relative speices are also important to conduct to better understand the system.

General discussion

1. Level of inbreeding potentiality in parasitoids: sex determination, Local Mate Competition and host distribution are linked.

1.1. Importance of the sex determination rule and the host exploitation strategies

In Hymenoptera species, the **sex determination rule** is of importance because it influences the sensitivity of a given species to inbreeding depression. Species carrying the single-locus CSD are very sensitive to inbreeding. Indeed, single-locus CSD introduces a high source of genetic load in small populations: the production of diploid males (often unviable or steriles, Heimpel & De Boer, 2008). Large haplodiploid populations can maintain diversity in CSD alleles and thus have low levels of diploid male production while small populations have less allelic richness and rapidly increase diploid male production (Zayed & Parker, 2005), which can induce population extinction.

Among parasitoid wasps, the mode of development (solitary, gregarious or quasi-gregarious) might play an important role in evolution away from the genetic load associated with *sl*-CSD. Outbreeding is expected to be more common in solitary species, whereas sib-mating is expected in gregarious species (Godfray, 1994; Godfray & Cook, 1997). High inbreeding levels in gregarious species would select for modes of sex determination that penalize inbreeding less than *sl*-CSD. For instance, this is the case in the Chalcoidea super family where no evidence of CSD had been found. Alternatively, if *sl*-CSD is retained, selection should favour other mechanisms to reduce the genetic load, such as avoidance of inbreeding or restoring reproductive function in diploid males (De Boer *et al.,* 2007).

For instance, the gregarious parasitoid *Bracon hebetor* has two behavioural mechanisms that reduce the probability of inbreeding as it has the CSD mode of sex determination (Ode *et al*., 1995). First, most, but not all, males and females are unreceptive to mating during the first 2 h after emergence. This time period is greater than the time necessary for individuals to disperse from the natal site. Second, females recognize and discriminate against males that develop at the same natal site as themselves. They retain this ability for at least 5 days (Ode *et al*., 1995).

Our experiments with *A. matricariae* are based on the initial assumption that *A. matricariae* has the same kind of sex determination as *A. rhopalosiphi*. In this species, iso-female lines rapidly generate an increase of the proportion of males (Salin *et al.,* 2004). Some preliminary results obtained on *A. matricariae* have shown consequences of inbreeding depression on various aspects affecting the female fitness. A decrease in female fecundity (nb of mummies obtained from one 24h egg laying period of <24h mated females) and an increasing proportion of males were observed after only 3 generations of brother-sister crosses (Brasseur, 2010). However, due to experimental problems (bacteria infestation of the aphid diet in some iso-female lines that caused mortality of aphids), we do not have enough data dot statistically confirm our observations. However, it is now **important to precisely investigate the sex determination rule and the consequences of inbreeding depression of *A. matricariae*** and other *Aphidius* species because of the following reasons:

1) "The *Cotesia* case". Species of the *Cotesia* genus are parasitoids of Lepidoptera larvae. S*l*-CSD is absent in the gregarious species *C. flavipes* and *C. sesamiae* (Niyibigira *et al.*, 2004a,b) but present in the solitary *C. rubecula* (Stouthamer *et al.* 1992) and *C. vestalis* (DeBoer *et al.,* 2006). In addition, Zhou *et al.* (2006) recently demonstrated CSD in the gregarious *C. glomerata*, confirming the absence and presence of CSD within a single genus for the first time. Both *Cotesia* species in which CSD is absent are gregarious, and two of the three species that have CSD are solitary, which suggests that the mode of development (solitary *vs*. gregarious) may indeed play a role in the evolution of sex determination in this genus (De Boer *et al.,* 2007).

The genetic load associated with *sl*-CSD in gregarious species can be relieved in one or more of the following ways: (1) avoidance of inbreeding (Ode *et al*., 1995); (2) restoring reproductive function in diploid males (Holloway *et al*., 1999); or (3) evolution away from *sl*-CSD. Inbreeding levels indeed appear to be low in *C. glomerata* despite gregarious development (Gu & Dorn, 2003). In the field, many males and most females leave the emergence patch directly after eclosion, and only 28 % of all females mate with a male from the same brood. In contrast, very high levels of inbreeding are common in *C. flavipes* and *C. sesamiae* (Arakaki & Ganaha, 1986; Niyibigira *et al.*, 2004).

The "*Cotesia* case" makes the comparison between species of the same genus indispensable to understand the evolution of mating system and mating strategies in parasitoids. This should be done both within the *Aphidius* genus and within aphid parasitoid species because they have different life history strategies. For instance, *A. ervi* is a low resource user (Schwörer & Volkl; 2001) because females attacked less than one third of suitable *A. pisum* hosts. In contrast, *Lysiphlebus hirticornis* is a specialist parasitoid and is described as a high resource user (Mackauer & Volkl, 2002;

Nyabuga *et al.,* 2010), since they parasitize many more aphids per colony. Recently Nyabuga et al. compared the mating strategies of these two species and found that nearly 90% of *L. hirticornis* mate on the natal patch (defined in this study as the whole plant) while only 20% of *A. ervi* do. In the same study they observe that the time interval between eclosion and first mating was lower for *L. hirticornis* than *A. ervi*. These two genuses are closely related, as they are closer together than with *Ephedrus* species.

2) The second aspect is linked to the consequence of inbreeding depression. Inbreeding depression does not always mean a rapid observation of diploid males in haplo-diploid species. Brother-sister mating could have other consequences than the apparition of a male biased sex ratio with many of diploid males. The consequences of inbreeding depression could appear through a lower fecundity of females, or any modified behaviour. Significant inbreeding depression in morphological, physiological, and social traits was first reported for honeybee, *Apis mellifera* (Bruckner, 1978). In another social Hymenoptera species, *Bombus terrestris*, comparison between full-sib and non-sib mating colonies showed no significant inbreeding effect on immune response or body size for workers or haploid males (Gerloff *et al*., 2003). Experimentally inbred female wasps in *U. semifumipennis* (Hymenoptera, Trichogrammatidae) suffer a 38% reduction in longevity (Henter, 2003). In this species, the reduction of fecundity is about 32% in inbred lines compared to outbred lines. No inbreeding depression towards parasitoid size occurs. This seems to be a general trend as morphological traits suffer little or no inbreeding depression compared to other life-history traits (DeRose & Roff, 1999). In the inbred lines we obtained for *A. matricariae*, we observed decreased fecundity with high levels of inbreeding, but, as stated before, no statistical analyses had been done because of the low number of replicates (Brasseur, 2010). In the near future, the consequences of inbreeding should be explored in *Aphidius* species, with particular attention given to fitness traits of both sexes (female fecundity, male sterility, behavioural traits such as host searching or response to sex pheromones, etc…).

The **female host exploitation** strategy and the sex-ratio allocation could provide us with an important understanding of the mating strategies of a quasi-gregarious species. The level of **Local Mate Competition** perceived by the female when it is laying its egg in a host patch can affect the sex-ratio. The understanding of the decision rules could help us to better understand the inbreeding avoidance mechanisms used within a species. Unfortunately the evolution of sex-ratio and sex allocation strategies were not investigated in this study and no data exist in the literature on *A. matricariae*. This is a very important point that has been to be explored in the imminent future.

However, in all of our experiments, females were provided at least 100 aphids of a standardized size. When we let the female oviposit in this patch in a Petri-dish, the sex ratio ranged between 12% and 100% of males depending of the age of the female or its previous experience (data of paper 3). Twenty-four hour old mated females parasitizing a patch of 100 aphids produced a sex ratio that ranged between 20 and 65% of males, with a mean of 43.2% (n=23 females). This sex ratio is in accordance with the model of Local Mate Competition with partial local mating and inbreeding avoidance. However, further studies are now required to evaluate more precisely the evolution of sex-ratio in this species and in other *Aphidius* species.

Recently Nyabuga *et al.* (2012) proposed to classify mating system in aphid parasitoids into two types. Type 1 is characterized by dispersal from the natal patch and outbreeding. It is typical of species parasitizing few aphids in each colony such as *A. ervi*. Type 2 is characterized by a high degree of sib-mating and LMC on the natal patch. It is typical of species producing large clutches such as *L. hirticornis*. The two systems are not mutually exclusive but represent the extremes of a continuum (Nyabuga *et al.,* 2012). These authors established that there is a relationship between average brood size and the proportion of sib-mating (Nyabuga *et al*., 2012). Indeed, they do not take in account the system of sex determination, which should be done to improve the model. Moreover, they do not take in account the behaviour of parasitized aphids, despite its potential importance on the spatial localisation of mummies, as we observed in this work (paper 1).

In future experiments, it will be important to utlizea comparative approach of different aphid parasitoid species from different taxonomical groups (Aphidiini, Ephedrini and Praini), including various aspects of their life history traits such as the mechanism of sex determination, the patch exploitation strategy of females (number of parasitized host, sex ratio), the behaviour of the host, and the post emergence behaviour of parasitoids in relation with their mating strategies. Such data exist in the literature but I think the methods used to collect data are too different to have a correct interpretation.

1.2. Importance of the host dispersal

When studying quasi-gregarious species, it is very important to **take into consideration the behaviour of their hosts** because it affects the parasitoid behaviour after emergence, especially when the host is still mobile some days after parasitism. In quasi-gregarious parasitoids with non-mobile hosts, such as egg parasitoids, the mating system is close to the one observed in gregarious parasitoids. Females adjust the sex-ratio of the brood according

to the LMC rules and favour a high female-biased brood to decrease future competition between their sons (Hardy, 1994). The sex ratio could be less female-biased in case of more females present on the host patch (Hardy, 1994). In these species, high levels of inbreeding often occur, but some exceptions test the rule, especially in the case of species that suffer from sib-mating (see precedent section). In quasi-gregarious species with mobile hosts, typically aphid parasitoids, the level of LMC should be relatively lower because of the potentiality of host dispersal. However, despite of its importance, few studies take it in account to study the mating strategies of these species (Nyabuga *et al*., 2012; He & Wang, 2008).

Aphid reaction towards a predator or parasitoid disturbance could be divided in two temporal steps that modify the spatial pathway and could influence the parasitoid spatial emergence.

1) First, the behaviour of the foraging predator or parasitoid induces a **rapid aphid reaction,** often resulting in specific dispersal behaviour.

The behavioural repertoire of disturbed aphids includes synchronized twitching, kicking, smearing with cornical secretion, walking away, and dropping from the plant (Pickett *et al.,* 1992; Wu *et al*., 2010; Vandermoten *et al*., 2012). Such behaviours can be initiated either by direct contact with a predator or a parasitoid, by sensing plant-borne vibrations caused by a foraging predator or parasitoid, by detecting an alarm pheromone released by a disturbed conspecifics, or by a combination of these factors (McConnell & Kring, 1990; Kunert *et al*., 2005; Podjasek *et al.,* 2005; Losey & Deznno, 1998; Bayaa, 2008; Wu *et al*., 2010).

Furthermore, a vast array of other factors (e.g. plant quality, aphid density, predator size, and physical factors) can moderate or intensify the response in aphids (Evans, 1976; Brodsky & Barlow, 1986; McConnell & Kring, 1990; Stadler *et al*., 1994). For instance, in the absence of foliar-foraging predators (low risk of predation), *A. pisum* had a very low propensity to drop from its host plant, even when aphid densities were high (Losey & Denno 1998). *A. pisum* that defend against coccinellid predators using cornicle secretions obtain both a direct (Dixon, 1958) and an indirect fitness benefit (Mondor & Roitberg, 2004). Recently, Wu *et al*. (2010) observed that the act of smearing *A.rhopalosiphi* with cornicle secretions can be considered altruistic for the cereal aphid *Sitobion avenae*, because it does not reduce the actor's probability of being parasitized, but reduces the parasitoid's rate of oviposition in the colony kin.

In our experiments, we did not observe the behaviour of *A. matricariae* when attacking the aphid colony. However, it seems that aphids disperse from the leaf containing the colony quite rapidly after parasitoid attack because we observed that 6 hours after the parasitoid attack, only 40% of aphids were still on the initial leaf (paper 1). In a long term experiment,

we found both parasitized and unparasitized aphids on the different plants of the cage, suggesting that a proportion of un-parasitized *Myzus persicae* reacts to the emission of alarm pheromones and disperses from the colony (Muller, 1983; Braendle & Weisser, 2001). As we did not observe the behaviour of the parasitoid female, it is possible that female contact with the aphid (with or without sting) triggered their dispersal and that the reaction towards the alarm pheromone is the only factor explaining the aphid dispersal.

However, we know that even if *Myzus persicae* respond to the EβF by dispersal behaviour (Francis *et al*., 2005), this species could be considered a quite immobile aphid when compared to other species such as *A. pisum* (McAllister *et al.,* 1990). We chose *Myzus persicae* as a host as we previously thought that the quite immobile behaviour of aphids should increase the propensity of *A. matricariae* to live quasi-gregariously. However, we found that parasitized *Myzus persicae* disperse, probably by walking. This confirms some cases of anti-predator behaviour of *Myzus persicae* (walking and drop of the plant) that had recently been observed against coccinellidae and sirphid predators (Belliure *et al*., 2011). It would be interesting to compare our results with other parasitoid or predator species on the same aphid. For instance, we know that *A. ervi* and *A. matricariae* are aggressive, their stings are rapid, and aphids react strongly by kicking, cornicules secretion, or walking. On the other hand, *Ephedrus cerasicola* is less aggressive, its sting is longer, and aphids react less (Dumont *et al.,* 2011).

In conclusion, **both predator and parasitoid behaviour act to favour the dispersal of a colony of aphids** and this aspect has to be taken in account when we want to understand the mating structure of parasitoids. The relation between aphid predators and parasitoids must be studied to understand how predator foraging behaviour can affect the parasitoid mating structure by increasing the dispersal of aphids but also through direct predation of parasitized aphids (Taylor *et al*., 1998; Colfer & Rosenheim, 2001). This rapid aphid dispersal could act indirectly to decrease the level of LMC of aphid parasitoids by increasing the spatial scale of emerging sibling parasitoids.

2) Second, aphid behaviour can change **some hours before mummification,** and is assumed to be under the control of the immature parasitoid (Boivin & Brodeur, 2004).

Poulin *et al*. (1994) hypothesized that koinobiont parasitoids should only manipulate the host when necessary, such as at the moment of a period of high vulnerability, i.e., metamorphosis. Authors working on host-

parasitoid systems identified two types of altered host behaviour: change in activity level and changes in habitat preference (Boivin & Brodeur, 2004).

One study of a cereal aphid (*Sitobion avenae*) has recently shown that parasitized aphids are significantly repelled by conspecifics a few hours before mummification (Guerra *et al.,* 1998). Other studies reported that parasitized hosts cease feeding and leave the aphid colony to mummify at specific sites (Boivin & Brodeur 2004). Aphid mummies are often found on the upper leaf surface or off the host plant (Behrendt, 1968; Brodeur & McNeil, 1989, 1990, 1991, 1992; Höller 1991, Müller *et al*., 1997). For instance, the survival of non-diapausing parasitoids is higher on upper surface of apical leaves rather than close to the aphid colony due to reduced level of parasitism and lower predation (Brodeur & Mc Neil 1991, 1992).

In our model, the architecture of the plant does not allow us to test the effect of the distance from the ground on the probability to find mummies, but we always observed mummies on the lower side of the turnip leaf. Experiments in progress with *A. ervi* parasitzing *M. persicae* or *A. pisum* on *Vicia faba* tend to confirm the hypothesis of aphid dispersal resulting in mummies placed on the upper leafs of the plant (Capella & Bourdais, preliminary results). We observed that when *Aphidius matricariae* females exploit a colony of *Myzus persicae*, aphids disperse and parasitoid offspring often emerge "alone" in the environment, meaning not in clumped patches of mummies. The probability to emerge alone on a turnip leaf is dependent of the size of the colony (paper 1). Moreover, we show that parasitoid males never induce the dispersal behaviour. The parasitoid females' behaviour is the main factor inducing the dispersal of aphids (paper 1). However, we need to be careful about our interpretations because of the patch problem, as discussed in the introduction.

To conclude, the observed aphid dispersal could result in **two non-exclusive results that favour on the one hand a low level of LMC and on the other hand decreasing the parasitoid probability to mate with a sibling.** First, a host-mediated behaviour occurring at a rapid temporal scale is probably due to the attack of the aphid colony by the female parasitoid. This induces aphid dispersal, which could also be due to other factors in natural conditions such as a predator foraging in the aphid colony. Second, a parasitoid mediated behaviour occurring before the parasitoid mummification, which is known to favour the selection of microhabitats favouring the survival of the mummy. However, other factors should be tested to better understand the influence of aphid dispersal on the parasitoid mating system, such as the size of the aphid colony, the distances between two colonies, and of course the scale of a patch for a mate searching parasitoid.

2. Some mechanisms of inbreeding avoidance in aphid parasitoids

2.1. Traits that might decrease sib-mating in aphid parasitoids

2.1.1.Emergence rhythmicity and dispersal

Rhythmicity in life-events such as mating, oviposition, hatching, moulting, pupation, emergence, foraging, and flight activity is well known in insects (Saunders, 2002; Myers, 2003). Activities requiring a circadian regulation are controlled by an endogenous clock, whose function has been characterized in various organisms (Bell-Pedesen *et al.,* 2005). In most cases, hatching is gated by the endogenous circadian system to a restricted period of the day (Myers, 2003) and individuals that reach maturity outside the gate emerge at the next following gate, usually 24h later. In insect species, emergence displays species-specific characteristics and could be linked to the mating system.

The temporal patterns of adult emergence have been observed for some parasitoid species and are more or less synchronized. However, emergence rhythms should be discussed in function of not only the environmental factors, but also the mating strategy of the species relative to inbreeding avoidance.

Rhythms in adult emergence are thought to be adaptive in insect species and one can ask **why most adult emergence occurs in the morning**? It was suggested that hatching in *Drosophila* happens in the early morning hours because the conditions of dawn facilitate freshly emerged flies to spread their wings (Kopik & Pittendrigh, 1967). We observed a peak in emergence during the first hours of the day in *A. matricariae* which probably resulted from the same reason (paper 2). If emergence from the pupal integument does not follow a circadian rhythm, hatched wasps may have to wait up to 24h inside the host puparium before emerging (Bertossa *et al.,* 2010; Karpova, 2006). This could explain why we did not observe any emergence of *A. matricariae* during the scotophase or the peak of emergence of males and females in the second and third mornings of the whole pattern (paper 2). Further observations are needed to determine when wasps are nearly emerging or waiting for optimal conditions inside the host chorion.

Moreover, if wasps, especially females, wait a few hours before emergence, it would be interesting to evaluate if this waiting period has a cost. Individuals that delay emergence may experience either an increase or a decrease in fitness, depending on how host resources are used. An increase in fitness is expected if the supplementary developmental time can be used by the individual to either fully exploit the host resource or to mature (Doyon & Boivin, 2005). In *A. matricariae* females, waiting for a few hours can allow them to mature and become receptive to mating just after emergence. Indeed, we observed that around 20% of females are able to mate just after emergence but we did not check if these females originated from the 1st day of emergence or the second day (paper 4). On the other hand, a decrease in fitness is more likely to occur if the individual is merely waiting for emergence while burning reserves to maintain its somatic functions. This aspect can be tested in *A. matricariae* males and females by evaluating the fitness of both sexes. Moreover, this aspect is important to evaluate in regards to the inbreeding avoidance mechanism of the species. Indeed, if males and females emerge together, a difference in the sexual maturation time could promote outbreeding (see the following section).

When we observe insect emergence rhythms, we often conclude that males emerge before females. **Protandry** occurs when males arrive at breeding areas on average earlier in the season than females (Morbey & Ydenberg, 2001; see introduction part).

If the difference between male and female emergence has fitness consequences for males or females, selection directly maintains sex-biased timing. For example, protandry may allow polygynous male butterflies to maximize mating opportunities with monogamous females (e.g. Wiklund & Fagerstrom, 1977; Iwasa *et al.,* 1983). In parasitoids, protandry associated with a low level of premating dispersal could be selected to favour in-patch mating in some species non sensitive to inbreeding. *Trichogramma* are short-lived species (Boivin & Lagacé, 1999), for which dispersal capacities are quite low (McDougall & Mill, 1997), and that mate mostly between relatives on the emergence patch (van den Assem *et al.,* 1980; Martel & Boivin, 2004). In this case, the timing of emergence is very synchronized (Doyon & Boivin, 2005; Reznik *et al*., 2008) to favour rapid mating of both sexes before dispersal, as mating between sibs does not have lethal consequences. In other species, the timing is less synchronized. In *Mellitobia sp.,* a quasi-gregarious parasitoid with immobile hosts (larvae of the saw-toothed grain beetles) most males hatch in the vicinity of their sisters and males emerge one to 2 days earlier and enter their sister's cocoons for mating (Powell, 1938; Collatz *et al.,* 2009). However, because unmated females produce isolated sons, males sometimes have to disperse to find a mate.

Protandry may also be a strategy to **facilitate outbreeding** in insects (Wedell, 1992). This idea is similar to protandry as a selecting avoidance strategy in plants (Richards, 1986). By dispersing from a common rearing environment into reproductive phase before nearby related females, males may increase the likelihood of mating with unrelated females. In *A. ervi*, the timing of emergence is similar to the one we observed in *A. matricariae* (He *et al.,* 2004), probably because they share common life history traits. In the two studied species, male and female emergences overlap even if on average, males have a shorter development time than females (He *et al*., 2004, the present study: paper 2). However, many males emerge slightly before females in the morning before the overlapping of emergence of both sexes. Thus, a certain proportion of males are effectively protandrous within a brood (males that emerge on day 1), while others are not because they emerge at the same time as females (males of day 2 or later males of day 1). These two strategies could favour an early dispersal of early males to other mummification patches. Competition for mates within a patch thus might occur between incoming males that have dispersed from another patch and late-coming males that emerge at the same time as females, favouring outbreeding. These hypotheses have to be explored in lab and semi-controlled conditions and the protandrous status of aphid parasitoids has to be considered in relation to their mating strategies. For instance, real conditions of light during dawn may change the pattern of emergence we observed in laboratory condition where the light was just on or off without an intermediate.

Nevertheless, the pattern of emergence observed in aphid parasitoids fits better with a solitary emergence of males and females than a classical quasi-gregarious one, as for instance in *Trichogramma*. In our experiments, we choose to test big populations of parasitoids (more than 400 standardized mummies), which could never happen in natural conditions within the same patch (also because of aphid dispersal, see paper 1). We thus supposed that aphid parasitoids mostly emerge in a more solitary way than quasi-gregarious parasitoids of immobile hosts and have to disperse to find a mate.

This idea has to be confirmed by studying field collected patches of mummies to better clarify how males and females emerge in natural conditions and confirm the "solitary aspect" of their mating system and the low level of LMC. Only field or semi-controlled trials in green houses could provide accurate answers, with genetic studies on the relatedness between spatially close mummies.

2.1.2. Sexual maturation

A difference in the timing of sexual maturation between sexes could act to reduce the Local Mate Competition and inbreeding levels if brother and sister emerge at the same moment and in a small spatial scale.

As mentioned in the introduction, few studies have been done on the sexual maturation of parasitoids, males and females being traditionally used at 24h old. However, a post-emergence refractory period may lessen the risk of mating between siblings (Antolin & Strand, 1992). He *et al.* (2004) showed that *A. ervi* females do not need a sexual maturation period but males need 4 hours to be able to mate. However, Nyabuga *et al*. (2012) observed an on-patch mating in *A. ervi* within 1.5 hours after emergence. This study also reported that most of the mating occurring on the natal patch was between local females and incoming males (Nyabuga *et al*. 2012). This reinforces the evidence for low LMC and low sib-mating in *A. ervi* due in part to the differential sexual maturation time between males and females. The differences we observed for *A. matricariae* could indicate the same conclusions except that it is the contrary. Females stay longer on the patch and probably wait for a male, either incoming males or newly emerged males. However, a differential sexual maturation time is not always necessary. In *Bracon hebetor*, rapid dispersal associated with 2 hours of sexual maturation in both sexes acts to decrease sib-mating on the natal patch. Oppositely, *Trichogramma* does not need sexual maturation time to favour on-patch mating before dispersal.

We do not have much data about the sexual maturation period of parasitoids. In other organisms, a sexual difference in maturation time exists for a many species but it is rarely linked with inbreeding avoidance (Pusey & Wolf, 1996). In the sub-social spider *Anelosimus* cf. *jucundus* (Bukowski & Aviles, 2002) authors suggest that these temporal patterns may limit the opportunities for sibling males and females to mate with each other, thus explaining the apparent absence of mechanisms to discriminate against kin as mates in this species. Putting forth evidence of a slightly delayed sexual maturation time requires a very sensitive time scale. However, future studies on the sexual maturation time of both sexes should be done in parasitoids as it could play an important role in inbreeding avoidance and mating strategies of a species by being one of the barriers against inbreeding.

2.2. Traits that might increase sib-mating

2.2.1. Number of mates in females

In most animal species, female polyandry is not selected directly to avoid inbreeding consequences but rather to increase the genetic diversity of offspring. In that way we speak more about extra-pair copulations, for which

the relatedness is not the only factor of choosing an extra-pair partner (Foerster *et al*., 2003; Pusey & Wolf, 1996). In many insect species, females mate several times, either with different males (polyandry) or with the same male (repeated mating) (Jennions & Petrie, 2000). Benefits of multiple mating for insect females might arise through different well-known pathways: (1) replenishment of depleted or unviable sperm (Lopez-Arroyo *et al.,* 1999; Drnevich *et al.,* 2001; Kraus *et al*., 2004), (2) transfer of nuptial gifts and nutriments (Eberhard & Cordero, 1995; Wiklund *et al.,* 2001; Gillot, 2003), (3) increase in the genetic diversity of the offspring (Yasui, 1998; Jennions & Petrie, 2000; Simmons, 2001), (4) avoidance of fight costs in case of male harassment (Rowe, 1992; Shuker & Day, 2002). However, mating and copulation could be costly in terms of time, energy, higher predation rate, or transfer of toxic substances and pathogens (Yasui, 1998; Arnqvist & Nilsson, 2000; Arnqvist & Rowe, 2005; Maklakov *et al.,* 2005; South & Lewis, 2011). The selection for monoandry or polyandry in females thus results from the expression of a trade-off between benefits and costs for a given species.

In a well known comparative study, Ridley (1993) concluded that solitary parasitoid species are mostly monoandrous and gregarious species are polyandrous.

Polyandry in gregarious and quasi-gregarious parasitoid species might reflect an adaptive strategy to minimize the risk of mating with males that have already emptied their sperm bank or to accumulate sperm from several partially sperm depleted males (*Trichogramma* species, Jacob & Boivin, 2005). It can also increase the probability of non-sib mating and increase the genetic diversity of the offspring if host patches have been exploited by several females. These two hypotheses are valuable when the emergence of males and females are simultaneous.

In fact, being monoandrous or polyandrous in quasi-gregarious species depends more on their emergence pattern and if they mate on the natal patch or disperse. In quasi-gregarious parasitoids where both males and females mate on the natal patch (such as *Trichogramma sp*.), females are polyandrous. In other quasi-gregarious parasitoids where males and females do not emerge in a close period of time, the proportion of multiple matings and male sperm depletion is probably less common. In that case, females tend to be monoandrous because one mate is enough to supply a sufficient amount of sperm (Arnqvist & Andres, 2006). Aphid parasitoids are known to be monoandrous. However, recently, some studies found that *Aphidius* females can accept to mate twice under laboratory conditions (Mc Clure *et al.,* 2007; He & Wang, 2008; this study: paper 3), suggesting that they are not as strictly monoandrous as we thought. If females emerge within a high male biased sex-ratio, they can mate multiply (He & Wang, 2008) and maybe gain genetic benefits by favouring unrelated mates. However, because they often emerge alone or with few males, they mate once because

one mate is enough to supply a sufficient amount of sperm (He, 2008; this study paper 3). These scenarios now must be tested, and the results could explain why some females mate twice in apparently monoandrous species.

Studies of the mating behaviour and courtship could help us to understand the proximate causes of polyandry in *Aphidius* females. We controlled most of the parameters that could influence mate acceptance in our experiment such as size, age, or mating status of the male, but we did not control for the relatedness of males and females because we randomly took males and females for rearing in the lab. Brothers and sisters do not avoid sib-mating (paper 5) but it is possible that the female could be able to evaluate the level of kinship and re-mate if she had the probability to find another unrelated partner (Välimäki *et al.,* 2011). In the same approach, it could be interesting to study the response of females previously mated with males of bad quality (small, old, or sperm depleted for instance) towards a new high quality mate. Indeed, in many insect species, females are able to evaluate the quality of the male and either refuse or accept any mate, but to use cryptic choice to use the better sperm to fertilize their eggs. Nevertheless, as discussed in paper 3, *Aphidius matricariae* females probably mate once in natural conditions and the species should be more characterized by being monoandrous than polyandrous.

2.2.2. Behavioural kin avoidance

One of the most commonly known ways to avoid mating between siblings is direct kin recognition and avoidance as a potential mate. As mentioned in the introduction of this work, experimental studies in a variety of species demonstrate that close relatives are often unattractive as mates (Pusey & Wolf, 1996). Mate choice in relation to kinship has poorly been investigated in insect species. However, kin recognition is often studied in insect such as social hymenoptera (Nehring *et al*. 2011), cockroaches or even in parasitoids (Lize *et al*. 2010).

The evolution of sib-mating in parasitoids depends on the mating system of the species and more precisely on its emergence patterns (solitary, quasi-gregarious, or gregarious) and the tolerance to inbreeding depression (Ode *et al.,* 1996; Reece *et al.,* 2004; Gu *et al.,* 2003; Abe *et al.,* 2003; Martel *et al.,* 2008). Related to the mating system and the sex determination mechanism of *Bracon hebetor*, Ode *et al.* (1995) found that females tend to avoid mating with brood mates when given a choice between two males. Studies on *Nasonia vitripennis* and *Cotesia glomerata* showed that females did not seem to discriminate between sibs (Reece *et al.,* 2004; Gu & Dorn, 2003). In *Trichogramma* species, no sib-mating avoidance had been shown (Martel *et al.,* 2008); in this species brother-sister matings are probably common because of the on-patch mating following emergence. The

information about the **non-avoidance of sib mating** in *A. matricariae* (paper 5) is completely new for aphid parasitoids. The evolution of active behaviour to avoid sib-mating could happen if sib-mating has deleterious effects on the offspring and if the probability to encounter and mate with a sib is high. In our model, we did not find a behavioural evidence of a sib-mating avoidance, which does not mean that they do not perceive the difference between sib and non-sibs. The sensitivity of *A. matricariae* to inbreeding and brother-sister mating can affect the sex-ratio and the mortality rate after two generations of inbreeding (Brasseur, 2010), while inbreeding effects have been shown for closely related species (Salin *et al.,* 2004).

2.3. Optimisation: a way of interpretation of such different traits in aphid parasitoids?

Optimality foraging models predict optimisation of an organism's behaviour in order to maximise their lifetime fitness gain. Foraging for resources (food, mate, etc.) is well suited for optimality approaches as organisms should weigh foraging costs (in time, energy, risks) against potential gains. Optimal foraging has thus become an important paradigm in our quest to understand animal behaviour (Stephens & Krebs, 1986). In insect parasitoids, optimal foraging models have been applied mostly to host exploitation. Female parasitoids also allocate the sex of their progeny based on competition among mates (Hamilton, 1967), host quality (size, species, and sex) (Charnov, 1979; Charnov *et al.*, 1981), position in oviposition sequence (Suzuki *et al.*, 1984; Wajnberg, 1993), or population sex ratio (Rotary & Gerling, 1973; Werren & Charnov, 1978).

Thus, according to the optimization theory, individuals should select the optimal behaviour at any point of their life to optimize their fitness. Ageing acts as an important factor. Decisions relative to mating are under different selective forces and many intrinsic and extrinsic factors will modulate the optimal choice for both sexes.

Mate choice is an important evolutionary process that imposes sexual selection on the other sex and sometimes accounts for spectacular ornaments that would otherwise remain unexplained by natural selection (Darwin, 1871). How and why mate choice evolved has been vigorously debated in the past 25 years (Kokko *et al.,* 2003), especially because mate choice can be cryptic and could appear in females after insemination (Parker, 1971). Obviously, the costs of searching for and discriminating among partners (costs of being choosy) and the costs of mating with any potential partner (costs of not being choosy enough) are important in the evolution of mate choice.

Mate choice in parasitoids has poorly been investigated except for one female trait: the mating status. In almost all species studied, a clear preference for virgin rather than mated partner was recorded (King *et al.,* 2005; Allen *et al.,* 1994; McNeil & Brodeur, 1995; Schworer *et al.,* 1999; Carazo *et al.,* 2003; Wedell *et al.,* 2002; Martel *et al.,* 2008). Male mate choice in relation to the female mating status is often mediated by chemical cues (sexual pheromones) (e.g. Carazo *et al.,* 2003; King e*t al.,* 2005) that allow the male to evaluate the mating status of the female. The influence of adult size on mate choice is less studied in parasitoid species but it seems that parasitoids prefer larger mates (Eggleton, 1990; Joyce *et al.,* 2009). The preference for a larger partner is mostly due to the heritability of the trait (Ellers *et al.,* 2001) although adult size can also be influenced by other factors (e.g. host size; Joyce *et al.,* 2002).

Ageing, the process of somatic deterioration that occurs as individual ages, is typically manifested in declining survival, reproduction, and reproductive value (Partidge & Barton, 1996; Bonduriansky & Brassil, 2005). During the last 10 years, we have observed a considerable interest in the role of ageing in sexual selection (Kokko & Lindström, 1996; Brooks & Kemp, 2001; Bondurianski & Brassil, 2005). In insects, many parameters, such as mating activities, are known to decline in both quantity and quality with advancing age (Rose, 1991). Studies on the effect of parental age on reproduction abound in insects (Mousseau & Dingle, 1991; Navasero & Elzer, 1992; Greenberg *et al.,* 1995; Foster & Howard, 1999; Maklakov *et al.,* 2007…). Different models have been proposed to explain the variation in mating success relative to age. In the first one, old age may signal 'good genes' for viability and females may evolve preferences for old males (Kokko & Lindström, 1996; Radwan, 2003; Nieberding *et al*., 2012). The model of Hansen & Price (1995) indicates younger males are more likely to be preferred mates while others suggest the selection of middle-aged males (Beck & Powell, 2000; Jones *et al.,* 2000).

In ageing experiments on *Aphidius matriariae*, males and females mate with old or young partners in the same proportion. However, they prefer young partners if they have the choice at the moment of mating. Parasitoid males can suffer from ageing by being less active or less attractive. They can also suffer from ageing if they are prospermatogenic (Boivin *et al.,* 2005). According to that index, males that emerge with their full complement of spermatozoids and do not produce more during their lifetime would be termed prospermatogenic (index=1). Inversely, males that mature spermatozoids later in life would be termed synspermatogenic (index=0) (Boivin *et al.,* 2005). We do not have data about the spermatogeny index of *Aphidius matricariae* males. However, the study of He (2008) on *A. ervi* males support the fact that they are medium synspermatogenic, meaning that they can suffer from sperm depletion in rapid successive matings but are able to produce new spermatozoids during their life. On the other hand,

mating at an older age in a monoandrous species, such as *Aphidius matricariae,* can be advantageous for the male because it decreases the mean competition rate within the whole population and increases their relative fitness (Damiens & Boivin, 2005). As in males, females can have a longer maturation time because of the longer period of gonad maturation (Majerus, 1994; Hodek & Hodek, 1996; Ravi & Palaniswami, 2002) while they also can suffer from ageing by having less viable eggs (Moore & Moore, 2001). However, mating when older could be advantageous for a hymenopteran female. Hymenopteran females can produce males through arrhenotokous parthenogenesis and females by fertilizing their eggs. Thus, even if a female had begun to produce males at the beginning of her life, mating at any stage of her life will allow her to produce females and increase her fitness more efficiently.

It appears that mating with a potentially poor quality partner is better than to not mate at all. Ageing and kinship experiments support our observations but, in *Aphidius matricariae*, males are not attracted at all by mated females (see paper 3 about monoandry). In this species, the mating status of the female seems to be the only reliable cue to explain the mate choice process. Despite these results, we observed that males often court all females they encounter and then stop if the females do not accept. We can ask the following question: Why do they spend so much time courting females or other individuals of the same sex, or even dead individuals? Courting, and attempting to mate with males or dead individuals is not new in insects (Steiner *et al.,* 2005; Dukas, 2010). However, we have to remember that these experiments were performed in Petri dishes, very far from a natural environment for parasitoids. These conditions could modify their behaviour. It is true that, in the field, females can more easily escape from the male and the amount of pheromones are diluted in the air. This was observed in ageing choice test experiments where old females were sexier for the young males (paper 4).

3. Mating system of aphid parasitoids: constraints, advantages, and impacts for the biological control of aphids

3.1. Aphid parasitoids: solitary or quasi-gregarious species? The problem of the patch still unresolved.

Aphid parasitoids are probably closer to a solitary species than gregarious species, but our interpretation depends of the notion of a patch.

Our results support the hypothesis that aphids disperse after a parasitoid attack, which favours a solitary emergence of offspring. However, we have to be careful about this notion of solitary emergence because we do not have experimental data or enough observation to concretely define a patch for a male searching for females.

As explained in the introduction and in the discussion of paper 1, a patch for a female is often considered as a spatial scale in which the female has a defined behaviour (for instance walking), and the female leaves the patch when it changes behaviour and flies away. It is the same definition in quasi-gregarious parasitoids with clumped hosts, such as *Trichogramma* species. However, aphids move from their initial place when parasitized or disturbed by a predator, which results in a larger spatial scale of emerged individuals. This is why we should evaluate the male and female post emergence behaviour of parasitoids and take into account the aphid behaviour and mummy localisation in field.

As aphid parasitoids seem to emerge alone, they have to deal with at least one major challenge: finding a mate. Many parasitoids use sexual pheromones that attract males (Mc Clure *et al*., 2007).

Solitary emergence could give advantages to the aphid parasitoid by decreasing the probability to mate with a sib. We still need additional data, but our experiment supports the fact that many aspects should combine together to decrease the sib-mating probabilities in Aphid parasitoids such as *A. matricariae*. Aphids disperse, which leads to a bigger spatial scale of emergence, brothers and sisters of the same brood do not emerge at the same time, sexual maturation, early dispersal… Obviously, the next step could be to model our behavioural observations to predict what could happen in different population sizes and further, how aphid population dynamics could

affect the parasitoid mating system. In a parallel way, fundamental research about the sex determination rules of Aphidiinae wasps are now essential to conduct in order to fullu understand if they are resistant to inbreeding.

3.2. Importance for biological control of aphids

In a **biological control context**, aphid dispersal and the solitary emergence of females could have huge consequences. In natural conditions, aphids are well known plant virus transmitters (Ng & Perry, 2004). Viruses are often acquired by the aphid during phloem ingestion from an infected plant and can be injected into another plant (Gildow, 1990). For the disease to spread through the plant population, however, there is also a need for the vectors to eventually disperse to new uninfected hosts (McElhany *et al*., 1995; Sisterson, 2008). Some studies have put forth evidence of the role of alate aphids in the propagation of viruses (Cheng & Feng, 2004; Feng *et al.,* 2007). In a recent study, Christiansen-Weniger *et al*. (1998) observed that aphids which carried the BYDV virus are not good hosts for *A. ervi*. It seems that parasitoid females are able to recognize aphids carrying a virus and avoid laying eggs in these aphids (Christianse-Weniger *et al.,* 1998).

However, if the parasitoid female exploits a colony with virus-infested aphids, all aphids could potentially disperse in response to the emission of the alarm pheromone (Vandermoten *et al.,* 2012) and inoculate the virus to other plants. For instance, if the predator causes escaping aphids to move between plants, this can result in an increase in dissemination of the pathogen throughout the plant population (Bailey *et al*., 1995; Weber *et al*., 1996). Hodge and Powell (2008) demonstrated that *Aphidius ervi* increased the dispersal of pea enation mosaic virus (PEMV) by *Acyrthosiphon pisum* (Harris) amongst peripheral plants of *Vicia faba* L. Indeed, disturbance by predators or parasitoids, directly or indirectly via alarm pheromones, can greatly affect the orientation and dispersal behaviour of aphids after they have dropped from the plant: aphids that have encountered predators tend to move more quickly after reaching the ground rather than employing thanatosis (‘playing dead’), move greater distances and change direction less, resulting in a reduced likelihood of re-settling on the original host plant (Phelan *et al*., 1976; Roitberg *et al*., 1979; Gish & Inbar, 2006).

The implications of the results obtained in the present study (paper 1) should also be considered when evaluating the use of beneficial insects in biocontrol strategies when an herbivore pest is also the vector of a plant disease.

General conclusions about parasitoids mating systems

The comprehension of the mating system of a species is more complex than traditionally presumed. Although different mating systems have long been recognized in evolutionary biology, there is far from universal agreement in their classification, and the often occurring terms are used in several senses. The number of mating partner per males and females is one of the main criteria used in classificiation, but there are other factors as well. Mating systems are related to resources necessary for breeding, such as the spatial distribution of food, mates, breeding areas, and the temporal distribution of mates. Moreover, mate choice of both sexes reflects various aspects of sexual selection and varies with time.

Understanding the mating system of parasitoids is potentially more complex than in other organisms because they depend on a host to achieve their development and because of the diverse sex determination rules that influence their resistance to inbreeding.

The relationship between the resistance to inbreeding and the mating strategies of parasitoids are complex and result also in a coevolution between the host and the life history traits of its parasitoid. As the spatial and temporal distribution of mates influence the mating system of any species and also parasitoids, the most obvious distinction made is between solitary parasitoids and gregarious ones. The level of gregariousness of a species can be described as the spatial localisation of individuals at the time of emergence and the level of kinship between them. Gregarious parasitoids are defined as species in which the female lays several eggs in a host, so that brothers and sisters will emerge and mate (LMC). Quasi-gregarious parasitoids are intermediate between solitary and gregarious because the level of gregariousness depends on the host behaviour and not only on the female patch exploitation strategy. Quasi-gregarious species with immobile hosts (egg parasitoids) are closer to gregarious ones than solitary ones. In contrary, quasi-gregarious parasitoids with mobile hosts may be more close to solitary species.

Recently, Nyabuga *et al.* (2012) proposed 2 types of mating system for quasi-gregarious parasitoids:

Type 1 is characterized by dispersal from the natal patch and outbreeding. Type 2 is characterized by a high degree of sib mating and LMC on the natal patch. These 2 types represent the extremes of a continuum, the position of each species on this continuum depends on its pattern of resource utilisation, the average clutch size but also the sensibility to inbreeding, the host behaviour or interactions with other organisms (ants, predators…). This categorization of mating systems of parasitoids takes in account the kinship relations of mates and could be generalized to other parasitoids. The principal factor in this definition is the relation of the species towards the risk of sib-matings. Fundamental research about the sex determination rules and the consequences of sib-mating in parasitoids are thus very important to conduct. It seems that independently of the clutch size

and the female host exploitation strategies or the gregariousness of a species, its resistance to inbreeding has selected various behaviours and mechanisms in different species.

Main results of the thesis

1. In lab conditions, *Myzus persicae* larvae disperse when attacked by *A. matricariae* females but the colony stays stable when only male parasitoids are present (paper 1).
2. The aphid dispersal leads to a solitary mummification of parasitized aphids in low density populations. However, the number of mummies clumped in patches can increase with higher densities of aphids (paper 1).
3. *A. matricariae* males and females of the same cohort (= groups of aphids parasitized during a close time interval) show a distribution of emergence during 3 days (paper 2).
4. The light triggers the emergence (paper 2)
5. *A. matricariae* seems protandrous but male and female emergence overlaps (paper 2)
6. *A. matricariae* females mate once most of the time (but can accept a second mate) (paper 3), are mature 30 minutes after emergence and are receptive until around 6 days old (paper 4).
7. *A. matricariae* males are receptive to mating just after emergence and for at least 10 days in lab conditions (paper 4).
8. No evidence for a behaviour avoidance of sib-mating was observed (paper 5)

To conclude, the results of this thesis propose a new way of thinking about the spatio-temporal patterns of dispersal of aphid parasitoids. Some of our data suggest that aphid parasitoids could not be considered a quasi-gregarious species but are closer to solitary species. Obviously, field investigations are necessary to confirm, because aphid populations vary within a year and under particular conditions, aphid parasitoids could behave as true quasi-gregarious ones. According to our results, we propose that *A. matricariae* does not present a high level of inbreeding in natural populations, but this should be tested in the field.

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Annexes

Adult size in males and females of the aphid parasitoid *Aphidius matricariae* : are latecomers bigger ?

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Insect developmental rates and emergence rhythms tend to be similar for all individuals of a population reared under the same environmental conditions.

However, variations in the developmental period of cohorts reared under the same conditions have been found in many species.

GOAL

In parasitoid species, adult size is influenced by the sex (females are usually larger than males) or the host quality (bigger is the host, bigger is the parasitoid) [1]

Adult size could have consequences on various traits in our lab studies, influencing for instance fecundity (number of eggs and spermatozoids), mate choice, thermal resistance, longevity …

Importance of size standardisation in experiments !

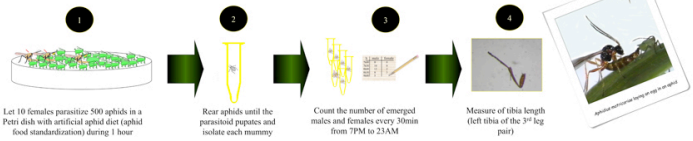
In this study, we wanted to test if variation in the developmental time in a cohort reared under the same conditions has an influence on adult size, using as model *Aphidius matricariae* (Hymenoptera : Braconidae).

Aphidius matricariae
Hymenoptera : Braconidae

- ✓ Small aphid parasitoid
- ✓ Generalist (parasitize 40 aphid species)
- ✓ Used in biological control programms
- ✓ Reared in our lab on *Myzus persicae* Sulz. (Homoptera : Aphididae), at 20°C±1°C.

What is a parasitoid?
An organism which develops on or in another single ("host") organism, extracts nourishment from it, and kills it as a direct or indirect result of that development.
Eggleton & Gaston 1990

METHOD

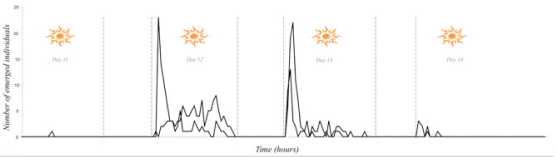


1. Let 10 females parasitize 500 aphids in a Petri dish with artificial aphid diet (aphid food standardization) during 1 hour
2. Rear aphids until the parasitoid pupates and isolate each mummy
3. Count the number of emerged males and females every 30min from 7PM to 23AM
4. Measure of tibia length (left tibia of the 3rd leg pair)

RESULTS

Spreading over time of emerged males (blue line) and females (pink line).

Number of emerged individuals observed in each 30min period from 7AM of the day 11 after aphid parasitization to 23PM of the day 14.



% of emerged individuals (n=number of individuals)	0.7 (n=1)	70 (n=89)	28 (n=36)	0.7 (n=1)
	/	52 (n=106)	43 (n=89)	5 (n=8)
Mean male tibia lenght (mm)	0.52	0.58±0.0052	0.57±0.006	0.5330
Mean female tibia lenght (mm)		0.61±0.005	0.61±0.009	0.63±0.02

Emergence of individuals from a same cohort reared under the same conditions spreads 4 days
Females are bigger than males *(GLM, F=15.81, p<0.0001)*
No influence of development time on adult size *(females : ANOVA, F=0.5, p=0.61 ; males : ANOVA, F=1, p=0.39)*

CONCLUSIONS

- *Aphidius matricariae* females are bigger
  - ✓ Sexual size dimorphism is current in invertebrates[2]
  - ✓ Insects have typically female-biased size dimorphism that is usually explained by the strong fecundity advantage of larger size in females [3]
- No influence of development time on adult size
  - ✓ Reject the hypothesis of selection of sexual size dimorphism due to protandry [3, 4]
  - ✓ Development time could have an influence on other traits
    - ✓ Adult longevity [5]
    - ✓ Reproductive traits (number of eggs or spermatozoids) [4,5]

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Annexe 1.

Publication list

Published

Bourdais D., Vernon P., Krespi L. Le Lannic J. and van Baaren J. 2006. Antennal structure of male and female Aphidius rhopalosiphi DeStefani-Peres (Hymenoptera: Braconidae): description and morphological alterations after cold or heat exposure. Microscopy Research and Technique 69(12):1005-1013

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Submitted

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