The effect of temperature, age and hunger on adult female fitness and on host-feeding behaviour in *Trichogramma* (Hymenoptera: Trichogrammatidae)

By Émilie Lessard

Department of Natural Resource Sciences
McGill University, Montreal

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ABSTRACT

The occurrence of sub-optimal temperatures during development of immature parasitoids can have important impacts on adult fitness. The effects of low temperature exposure on different fitness proxies were investigated in *Trichogramma brassicae* Bezdenko. The host-feeding behaviour, defined as the consumption of host heamolymph and tissue by the adult female, was measured as an indicator of energy reserve after low temperature exposure. A decrease in percentage of emergence, longevity and fecundity was observed and fitness costs increased with duration of exposure and temperature (combined effect). However, allowing development and acclimation at 13 or 10°C reduced fitness costs. Acclimation treatment at 10°C during 30 days resulted in the lowest decrease of fitness proxies. No difference in host-feeding frequency, host-feeding duration and number of hosts used for feeding was observed between the treatments with and without acclimation. Contrary to our prediction, host-feeding behaviour did not reflect the loss in energy reserve after low temperature exposure.

We tested the effect of age and hunger on females' host-feeding behaviour in *Trichogramma euproctidis* Girault. The distribution of host-feeding bouts in a sequence of 10 hosts encountered and the sex ratios deposited were also investigated. No difference in frequency of host-feeding, duration and number of hosts used was observed between young and old females. Starved females host-fed for a longer duration and were less mobile. The sex-ratio of the progeny produced on the first host parasitized was more male-biased when host-feeding occurred. Host-feeding had no effect on the sex-ratio deposited elsewhere in the sequence of 10 hosts encountered. The female's age had no effect on host-feeding, possibly because it incurs little fitness cost for this species. To

host-feed on the first host parasitized, in which a male is allocated, is less costly in term of fitness and represents a strategic choice for the female. Host-feeding may also be partly motivated by thirst, as access to water decreased the duration of host-feeding behaviour.

RÉSUMÉ

L’occurrence de températures sous-optimales durant le développement des parasitoïdes immatures peut avoir des impacts importants sur la valeur adaptative de l’adulte. Les effets de l’exposition aux basses températures sur différents estimateurs de la valeur adaptative ont été étudiés chez *Trichogramma brassicae* Bezdenko. Le comportement de nutrition sur l’hôte, défini comme étant la consommation de l’hémolymphe et des tissus de l’hôte par la femelle adulte, a été mesuré comme indicateur du niveau des réserves d’énergie après l’exposition aux basses températures. Une diminution dans le pourcentage d’émergence, la longévité et la fécondité ont été observées et les coûts en valeur adaptative augmentaient avec la durée et la température d’exposition (effet combiné). Par contre, permettre le développement et l’acclimatation à 13 ou 10°C réduisait les coûts en valeur adaptative. Le traitement d’acclimatation de 30 jours à 10°C donnait la plus petite diminution dans les estimateurs de valeur adaptative. Aucune différence dans la fréquence et la durée de la nutrition sur l’hôte, ainsi que dans le nombre d’hôtes utilisés pour se nourrir n’a été observée entre les traitements avec ou sans acclimatation. Contrairement aux prédictions, la nutrition sur l’hôte ne reflétait pas la diminution dans les réserves d’énergie après l’exposition aux basses températures.

Nous avons testé l’effet de l’âge et de la faim sur le comportement de nutrition sur l’hôte des femelles *Trichogramma euproctidis* Girault. La distribution des évènements de nutrition dans une séquence de 10 hôtes rencontrés ainsi que le ratio des sexes déposé ont également été étudiés. Aucune différence dans la fréquence, la durée de la nutrition sur l’hôte et dans le nombre d’hôtes utilisés pour se nourrir n’a été observée entre vieilles et jeunes femelles. Par contre, les femelles à jeun se sont nourries plus longtemps et étaient moins mobiles. La nutrition sur l’hôte était plus fréquente sur le premier œuf hôte rencontré et la proportion de mâles alloués sur cet hôte était plus grande. La nutrition sur l’hôte n’a pas eu d’effet sur le ratio des sexes déposé ailleurs dans la séquence de 10 hôtes rencontrés. Contrairement aux prédictions des modèles, l’âge de la femelle n’a pas eu d’effet sur la nutrition sur l’hôte possiblement parce que la nutrition engendre des coûts minimes en valeur adaptative chez cette espèce. Se nourrir sur le premier hôte parasité, dans lequel un mâle a été alloué, est moins coûteux en termes de valeur adaptative et représente un choix stratégique pour la femelle. La nutrition sur l’hôte peut être également partiellement motivée par la soif, puisque l’accès à l’eau réduisait la durée du comportement de nutrition.

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CONTRIBUTIONS OF AUTHORS

All chapters are co-authored papers submitted or to be submitted. I have done all the experiments and analysis, and written all papers. The co-author, Dr Guy Boivin, has revised and corrected the papers before their submission. The second chapter, “Effect of low temperature on emergence, fecundity, longevity and host-feeding by *Trichogramma brassicae*”, has been submitted to Biocontrol. The third chapter, “Effect of age and hunger on host-feeding behaviour by female *Trichogramma euproctidis*”, has been submitted to The Canadian Entomologist.

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INTRODUCTION

Introduction

When subjected to low temperature during its immature development, a parasitoid adult has reduced energy reserve and may suffer from different cold injuries (Chen et al. 2008). Low temperature can cause physiological dysfunctions (Colinet and Boivin 2011), depletion of energy reserves (Chen et al. 2008) and morphological alterations such as malformations of reproductive organs (Denlinger and Lee 1998), a reduction in body size (Rundle et al. 2004), wings deformity (Dutton and Bigler 1995, Tezze and Botto 2004) and alteration of antennal structure (Pintureau and Daumal 1995). It can also change behavioural decisions by the adults (Hance et al. 2007). At low temperature, immature development takes longer and more resources have to be allocated to metabolic maintenance at the detriment of other traits such as reproduction (Boivin 2010). These effects can be even more important in egg parasitoids such as *Trichogramma*, since they have limited resource. Furthermore, fitness usually decreases with temperature and duration of exposure (combined effect) (Colinet and Boivin 2011). While the intensity of the response varies between species, preconditioning individuals through acclimation at low temperature can improve parasitoid survival and increae the fitness associated with low temperature exposure (Pandey and Johnson 2005). When their energy reserve have been affected by exposure to low temperature as an immature, adult parasitoid females can replenish it by feeding on potential hosts.

The reproductive success of adult female parasitoids can be greatly affected by their diet and their energy reserve. The importance of finding food sources for their maintenance, survival and for egg maturation has been well documented (Jervis et al. 1996, Heimpel et al. 1997, Lewis et al. 1998). Adult females can obtain food both from other insects or from plants. Both males and females can obtain proteins and sugars directly from plant sources such as pollen and floral and extrafloral nectar or indirectly through honeydew (Jervis et al. 1992). These food sources however are not always available close to their hosts and acquiring these resources often imply some travel time (Jacob and Evans 2001, Bernstein and Jervis 2008, Jervis et al. 2008). Some females of parasitoid species feed upon other insects by consuming their hemolymph and tissues (host-feeding) (Jervis and Kidd 1986). Host-feeding decreases travel time out of the host habitat to search for other sources of feeding. The role of nutrients obtained from host-feeding appears to be mainly for egg maturation, although it can also be used for somatic maintenance in some species (Jervis and Kidd 1986, van Lenteren et al. 1987, Collier 1995, Heimpel and Collier 1996, Heimpel and Rosenheim 1995, Giron et al. 2004, Ferracini et al. 2006), but it is generally accepted that the role of non-host food is primarily to supply energy for somatic maintenance (Jervis and Kidd 1986, Thompson 1999).

Dynamic state-variable models have been developed to predict how different factors affect the propensity of parasitoids to host-feed. These models balance the risk for the parasitoid of becoming egg-limited versus time-limited in order to maximize lifetime reproductive success (Heimpel and Collier 1996). They predict that host-feeding should increase when the egg load of a female is low, when there is oosorption, when mortality risk is low, when nutrients reserves and gut contents are low, and when host quality is low. Models also predict that host-feeding should decrease at older age. These models' predictions have been tested empirically with various species of parasitoids (Rosenheim and Rosen 1992, Heimpel et al. 1994, Collier 1995), but have not been verified with an egg parasitoid doing concurrent and non-destructive host-feeding such as *Trichogramma*.

Parasitoids of genus *Trichogramma* are small hymenopterous egg parasitoids from the Trichogrammatidae family. This group has been studied extensively because of their efficiency in biological control (Smith 1996). The resources available for the developing immature is fixed as the host is killed or paralysed at the time of oviposition (idiobiont parasitoid) (Godfray 1994). Thus, idiobiont parasitoids are more constrained in total capital resource than koinobiont, where the parasitoid allows hosts to continue to grow in size after parasitism (Godfray 1994). *Trichogramma* wasps do concurrent but non-destructive host-feeding which mean that oviposition and host-feeding take place on a same host (concurrent) and that the host is still suitable for progeny development after the meal (non-destructive) (Jervis et al. 1996). The size of individuals developing in eggs on which a female host-fed is smaller; indicating that progeny's fitness is affected and host-feeding decreases the resources available (Ferracini et al. 2006). However, the fitness cost is small in *Trichogramma*, since host-feeding by the mother decreases by less than 5% the tibia length in progeny (Ferracini et al. 2006). The decision of a female to host-feed should takes into account their own condition and energy reserve, the gain obtained by host-feeding plus the consequence of that behaviour on their progeny fitness. Observation of the host-feeding behaviour could also allow us to measure indirectly the impact of low temperature on the energy reserve of the adult.

Objectives

The aim of this study was first to quantify the effect of low temperature exposure during immature development on several fitness proxies (survival, longevity, fecundity, sex ratio deposited, mobility) and on the host-feeding behaviour, used here as an indicator of energy level. The second part of the thesis examines the effect of age and hunger on female's host-feeding behaviour and on its mobility on a host patch. The obtained results will improve our understanding of the behavioural ecology of *Trichogramma* species and could be used to optimize mass rearing and biological control. Two species have been chosen: *T. brassicae* Bezdenko and *T. euproctidis* Girault. Both species have good potential in biological control.

Hypotheses

Four hypotheses will be tested in this study:

1- Females *T. brassicae* fitness should be affected with temperature and duration of exposure during their development. As temperature decreases and duration increases, fitness should reduce.

2- Acclimation and maternal short photoperiod should reduce the fitness cost associated with low temperature exposure.

3- Host-feeding can be used as an indicator of stress caused by immature exposure to cold.

4- Age and hunger should affect the host-feeding behaviour.

Thesis format

This thesis is a manuscript-based thesis. Each chapter has been or will be submitted to scientific journals as mentioned at the beginning of each chapter. The contributions of authors are mentioned at page viii.

CHAPTER I

Literature review

1. Effect of low temperature

1.1 Cold hardening

Definition

The term cold hardening refers to the physical and metabolic modifications that enable an organism to survive low temperatures (Leopold 1998). Cold hardening can be a long term process obtained after weeks or months at low temperature or a very rapid process within minutes or hours after exposure (Denlinger and Lee 1998). It may or may not be directly linked with dormancy. We often refer to acclimation and acclimatization when we talk about conditioning individuals to low temperature in the cold hardening process (Leopold 1998). We refer to "acclimation" when gradual physiological response to altered environmental conditions occurs in laboratory in contrast to "acclimatization" that occurs in nature (Withers 1992, Levie et al. 2005).

For insects, the challenge of surviving low temperatures is met with two main strategies: cold avoidance and cold hardiness (Boivin 1994). In the first case, insects may migrate to places where temperature can be tolerated or select a microhabitat that will protect them from adverse conditions. In the second case, a series of biochemical and physiological adaptations allow insects to tolerate prolonged exposure to low temperature. The damage caused by the freezing of intercellular fluids may be fatal for some insects while other can resist and even encourage the creation of ice inside their body at high subfreezing temperature. This is the major distinction between freezing-tolerant and freezing-intolerante insects (Salt 1961, Zachariassen 1985, Brown et al. 2004). Freezing-intolerant insects avoid ice formation by synthesizing cryoprotectants, by eliminating ice-nucleating agents, by not feeding before the cold period, by dehydrating or by increasing their solute concentrations (Salt 1961, Boivin 1994, Denlinger and Lee 1998). Freezing-tolerant insects, however, are adapted to accelerate ice formation via ice-nucleating agents present in the heamolymph or in the digestive system (Salt 1961) and usually freeze at about –5°C to –8°C (Boivin 1994).

1.2 Dormancy

Definition

Dormancy may be considered as part of the cold hardiness strategy used by insect to survive adverse conditions. However, dormancy is not restricted to insects that are overwintering and may be found as well in tropical and temperate zones during summer (Denlinger and Lee 1998). Two physiological adaptations allow insects to overwinter in a dormancy state: quiescence and diapause (Danks 1987). Quiescence is an immediate direct response to a limiting factor, such as when temperature falls below the insect's development threshold, but immediate resumption of development occurs when the conditions improve. Diapause is a more profound interruption of development and is a programmed response usually preceding the adverse conditions. Environmental factors, taken as cues by the insect, indirectly modify developmental pathways and the suppressed development can last longer than the adverse conditions (Danks 1987). Diapause is the natural response to long-term adverse conditions. Sometimes, it is difficult to distinguish between diapause and quiescence and distinguishing between the two has to be deduced from the pattern of emergence, the percentage emergence, or the time taken to complete development (Rundle and Hoffmann 2003).

Diapause does not necessarily imply cold hardiness and cold hardiness does not imply that the insect is in diapause (Denlinger and Lee 1998). Diapause can be a pre-requisite for cold hardiness but both phenomena can also be independent (Danks 2002, Hodkova and Hodek 2004). Environmental cues are used for both, entering in diapause and cold hardiness by the insect. However, individuals in diapause are generally more cold hardy than non-diapausing individuals (Hodkova and Hodek 2004).

Not every species can enter diapause or quiescence and among the ones who can, conditions to induce dormancy are often species specific and not always easy to reproduce in laboratory. Knowledge of an insect's ability to survive cold and how to induce dormancy have practical applications and help in determining the chance of an insect to become established in temperate climates.

Temperature and photoperiod induce dormancy

Temperature is well documented as the key factor inducing dormancy. For the insect able to overwinter, it is usually a temperature decrease that induces dormancy. The exposure period plays an important role in diapause induction and is on a par with the temperature (Denlinger and Lee 1998). Change in temperature can also accelerate diapause or quiescence termination (Boivin 1994).

The photoperiod is another cue used by the insect to prepare for the incoming cold period in nature, but its effect is often slight over one generation. However, the photoperiodic state of the prior generation can influence the induction of diapause among the progeny and it is part of what we commonly call the maternal effect (Smith 1996, Zaslavski and Umarova 1990, Pizzol and Pintureau 2008). A reduced photoperiod usually induces dormancy. Several studies relate the effect of photoperiod on diapause induction (Polgar et al. 1995, Milonas and Savopoulou-Soultani 2000, Reznik and Vaghina 2007, Pizzol and Pintureau 2008,) but its effect on cold tolerance of non-diapausing individuals had received little attention (Hance and Boivin 1993, Kim and Song 2000) and its link with quiescent insects needs further studies.

1.3 Acclimation

Definition

We refer to "acclimation" when gradual physiological response to altered environmental conditions occurs in laboratory in contrast to "acclimatization" that occurs in nature (Withers 1992, Levie et al. 2005). Acclimation is often used to prepare insects to incoming low temperatures by conditioning them with an intermediate temperature and it often alleviates cold injury (Denlinger and Lee 1998). However, acclimation can also have a cost and its global effect on fitness will depend on the overall balance between costs and benefits (Hoffmann and Hewa-Kapuge 2000, Rako and Hoffmann 2006). For example, preconditioning individuals by exposing them to low temperature close to their developmental threshold before exposing them to a lower temperature can improve the survival and fecundity (benefits) of the parasitoid *Anagyrus ananatis* Gahan but individuals have a lower fecundity (cost) compared with control (Pandey and Johnson 2005).

1.4 Fitness proxies affected by low temperature

Definition Fitness

Fitness is defined as the success of an individual in transmitting copies of its genes to future generations (Bolhuis and Giraldeau 2005). The phenotype of an individual is affected by the environment as well as by genes and the fitness of a given phenotype can differ across environments. Finess of different individuals within the same genotype is therefore not necessarily equal (Haldane 1932). Direct fitness refers to the fitness gained by an individual through the production of its own offspring while inclusive fitness refers to the sum of the direct fitness plus the indirect fitness gained by the reproduction of nondescendant relatives (Krebs and Davies 1997). Another research area where measures of fitness are used is in applied ecology and especially in biological control (Roitberg et al. 2001). Fitness can be used to assess insect's capacity to multiply and control a targeted pest. A population with high fecundity, emergence, sex ratio (percentage of female offspring), longevity, host preference for the target species, host searching activity and tolerance to local weather conditions will be defined as a parasitoid of "high quality" (Smith 1996). These traits are assumed to be ecologically important for parasitoids when released inundatively and other characteristics such as development rate, competitive ability and oogenesis are also important for inoculative releases (Smith 1996).

Link between low temperature and fitness

Exposure to low temperature can negatively affect the fitness of an individual insect. Reduced temperature can cause: a decrease in the rate of enzymatic activity; changes in tertiary structure of proteins and disassembly of polypeptide subunits, resulting in irreversible protein denaturation; depolymerization of cytoplasmic microtubules and alteration in cell membrane permeability (Denlinger and Lee 1998). Damages to the insect body can occur even above freezing temperature.

Low temperature injuries differ according to insect stages. When immature stages are subjected to low temperature, the fat reserves and subsequent survival of the emerging adult is reduced (Chen et al. 2008). Even at non-freezing temperatures, immature parasitoids that develop at sub-optimal temperature usually suffer major fitness costs (van Baaren et al. 2005, Hance et al. 2007, Colinet and Boivin 2011). Certain developmental stages may be more sensitive to cold than others. The age of *Anaphes* sp. larvae and duration of cold exposure have a major influence on its survival (Boivin 1994). In *Trichogramma* spp., the prepupal stage is less sensitive to low temperature than the larvae (Garcia et al. 2002). When adults *Apanteles galleriae* Wilkinson are exposed at 6°C during one week, the injuries are fatal and result in 85.27% mortality (Uçkan and Gülel 2001).

Most of the studies on the effect of low temperature exposure on insect's fitness were conducted on insect parasitoids, because of their importance in biological control programs (Pandey and Johnson 2005, Lepopold 1998, Colinet and Boivin 2011). Cold storage is commonly used by industries to synchronize production with the demands in the field and to conserve large stock of insects. Fitness proxies are also often used to estimate the quality of insect prior to their use as biological control agents.

Longevity

A decrease in longevity of adults after low temperature exposure of immatures has been observed in many parasitoid species such as *Trichogramma* spp. (Jalali and Singh 1992), *Anagyrus ananatis* Gahan (Pandey and Johnson 2005), *Aphidius colemani* Vierek (Colinet et al. 2007), *Trissolcus basalis* Wollaston and *Telenomus podisi* Ashmead

(Foerster and Doetzer 2006). These species survived cold exposure but suffered from a severe reduction in their life expectancy because of cumulative cold injuries (Colinet and Boivin 2011). A constant decline of adult longevity with duration of low temperature exposure can also be observed in *Trichogramma* sp. and *Aphidius picipes* Nees (Rundle et al. 2004, Amice et al. 2008, Ayvaz et al. 2008).

Fecundity

A reduction in females' fecundity can appear after low temperature exposure (Uçkan and Gülel 2001, Bayram et al. 2005, Levie et al. 2005, Pandey and Johnson 2005). The survival at low temperature for egg parasitoid immatures is often at the detriment of female's fecundity and other traits linked with reproduction (Boivin 2010). Malformations of reproductive organs can also appear after low temperature exposure (Denlinger and Lee 1998) since the reproductive organs are particularly vulnerable to low temperature effects (Denlinger and Lee 1998, Colinet and Boivin 2011). In the worst case, sterility is a consequence of exposure to cold as it has been observed in *Anagyrus ananatis* Gahan males (Pandey and Johnson 2005).

Sex ratio

Sex ratio can be affected in either the generation subjected to low temperature or in the following generation. In the generation subjected to low temperature, it is often measured as emerging sex ratio or differential mortality between males and females. Differential mortality has been observed in immatures *Gonatocerus ashmeadi* Girault where the proportion of males emerging after cold storage was higher than what is normally observed without cold storage (Chen et al. 2008). The developing haploid progeny of *G. ashmeadi* (males) were more cold-tolerant than the diploid progeny (females). The sex ratio of the following generation can be further modified towards more males as a consequence of male sterility in arrhenotokous Hymenoptera, where unfertilized eggs produce males only (Pandey and Johnson 2005). A drift in F1 sex ratio to more males has been observed in *Telenomus busseolae* Gahan with an increasing length of cold storage treatment (Bayram et al. 2005).

Mobility

Mobility of parasitoid females can be used as an indicator of their fitness and their efficiency in host searching and parasitism (Bigler 1989, Dutton and Bigler 1995, Pompanon and Boulétreau 1997, Tezze and Botto 2004, Suverkropp et al. 2010). For instance, when *T. evanescens* Westwood immatures are exposed to 4°C for 28 days, walking speeds decrease (Ayvaz et al. 2008). Similarly, when *T. nerudai* Pintureau and Gerding immatures are exposed at 4°C for 50 days, the flying ability of the resulting adults is affected negatively (Tezze and Botto 2004). Low temperature exposure can also caused wings deformity in *T. brassicae* and *T. nerudai* when immatures are exposed to low temperature (Dutton and Bigler 1995, Tezze and Botto 2004).

Behaviours

Low temperature may also affect behaviours related with foraging. For instance, low temperature of 4°C during prepupal stage affects the locomotor activity in parasitoid *Trichogramma nerudai*, resulting in a decreased ability to parasitize as adult (Tezze and

Botto 2004). Behaviours associated with host location and evaluation can also be modified by reducing the parasitoid's learning and discrimination capacities (Hance et al. 2007). When the immatures of the mymarid *Anaphes victus* Huber (Hymenoptera: Mymaridae) are exposed to 4°C for 3 to 12 weeks, the resulting adults have reduced capacities of learning external oviposition marks and also to optimize their patch time allocation (van Baaren et al. 2005).

Percentage of emergence and survival rates

Percentage of emergence after low temperature exposure is used to estimate the quality of insect stocks. Percentage of emergence is reduced when immatures of many insect orders such as Hymenoptera (Bayram et al. 2005, Özder and Saglam 2005), Lepidoptera (Johnson 2007), Coleoptera (Teshler et al. 2004), Diptera (Leopold 2000) and Heteroptera (Coudron et al. 2007) are exposed to low temperatures. Direct cold storage of adults can also be fatal. For example, cold storage of adults *Apanteles galleriae* Wilkinson stored at 6°C during one week resulted in 85.27% mortality (Uçkan and Gülel 2001). Exposure of *Xanthogaleruca luteola* Muller at –15°C during 24 hours resulted in more than 70% of mortality (Soudi and Moharramipour 2011). Survival depends on both the duration and the temperature of exposure (i.e. the cold dose) (Colinet et al. 2011). Insect that do not survive has zero fitness and insect that survive has a value that cannot be estimated. However, we obtain a fitness value for the group subjected to low temperatures by counting individuals that survive.

2. Host-Feeding Behaviour

Definition

Host feeding is the consumption of host hemolymph and tissues by the parasitoid adult female (Jervis and Kidd 1986).

2.1 Type of host-feeding

Some parasitoid females can both host-feed and oviposit on the same individual host (concurrent host-feeding) and others use some individuals for oviposition and other individuals for host-feeding (non-concurrent host-feeding) (Godfray 1994). Usually, females practicing concurrent host-feeding do not remove a large quantity of fluid from the host and may either consume the hemolymph exuding from the puncture made with the ovipositor or make an extra incision (Godfray 1994). We distinguish destructive from non-destructive host-feeders when the host-feeding meal renders the host unsuitable for progeny development (Jervis et al. 1996). Destructive host-feeding is generally non-concurrent. The majority of parasitoids observed are destructive and non-concurrent host-feeders (Thompson 1999).

2.2 Nutrients obtained and their use

Female parasitoids can obtain proteins and lipids by consuming the hemolymph and tissues of their hosts but also sugar and glycogen (Giron et al. 2002). Nutrients obtained from host-feeding appears to be used mainly for egg maturation, although it can be used for somatic maintenance in some species (Jervis and Kidd 1986, van Lenteren et al. 1987, Collier 1995, Heimpel and Collier 1996, Heimpel and Rosenheim 1995, Giron et al. 2004, Ferracini et al. 2006).

2.3 Other sources of feeding

Female parasitoids gain sugars and some proteins by using floral and extrafloral nectar, pollen, as well as honeydew produced by homopteran insects (Jervis et al. 1992). These food sources can be in a different habitat than their hosts and as a result females have to invest time and energy to reach these resources (Jacob and Evans 2001, Bernstein and Jervis 2008, Jervis et al. 2008). It is generally accepted that the role of non-host food is primarily to supply energy for somatic maintenance (Jervis and Kidd 1986, Thompson 1999). However, optimal reproductive success requires both host and non-host feeding (Heimpel et al. 1997).

2.4 Models and predictions on host-feeding

A simple analytical model and several simulations models have been developed by Jervis and Kidd (1986) for host-feeding behaviour (reviewed by Heimpel and Collier 1996). These models predict the optimal fraction of hosts to be used for host-feeding (Jervis and Kidd 1986). These models use the following assumptions: the important constraints are energetic ones, host-feeding provides the metabolic demand of energy but does not influence survivorship, the parasitoids do not face a random chance of mortality (the parasitoid’s lifetime is of fixed duration) and the number of eggs oviposited during a female’s lifetime is a direct measure of its fitness (it does not take into account the fitness of the offspring). Offspring fitness would have been a better measure of fitness, but it was outside the scope of the treatments for modelling host-feeding strategies in these models (Jervis and Kidd 1986). The models predict that the fraction of hosts devoted to host-feeding should decline as host availability increases, unless host availability falls below a critical level (Heimpel and Collier 1996). When hosts vary in quality, young stages are favoured for host-feeding since they give less fitness per oviposition (Kidd and Jervis 1991).

Several dynamic state-variable models that were developed to predict how different factors affect a parasitoid's propensity to host-feed have started to be tested empirically (Chan and Godfray 1993, Heimpel et al. 1994, Collier 1995, McGregor 1997). These models balance the risk for the parasitoid of becoming egg-limited versus time-limited in order to maximize lifetime reproductive success and have been reviewed by Heimpel and Collier (1996). These models shared five features: (1) The parasitoid lifetime is divided into short time steps during which there is a probability of a host encounter. (2) When a host is encountered, the parasitoid rejects, oviposits or feeds on the host (host-feeding is thus non-concurrent but a model with concurrent host-feeding exists (Heimpel et al. 1994)). (3) Host-feeding leads to changes in gut contents, nutrients reserves and/or egg load. (4) The sum of current fitness gains (oviposition) and expected future fitness gains, which depend on the state variables and probability of surviving the time step, must be maximized by the appropriate behaviour. (5) The parasitoid's age is included as a state variable.

In the simplest dynamic state-variable models, host-feeding has one function and it is to supply nutrients for egg production and maturation (Chan and Godfray 1993, Collier et al. 1994). These models predict that parasitoids should host-feed when their egg load is zero (Heimpel and Collier 1996). The models that incorporate a metabolic demand on the nutrient gain by host-feeding are called “resource pool models” and since the parasitoid’s survival depends on the nutrient reserves, host-feeding can occur at egg loads greater than zero, at a critical level (Heimpel and Collier 1996). Other models assume that host-feeding is used only for metabolism but not for egg maturation (Houston et al. 1992, Chan and Godfray 1993) and also predict that host-feeding will occur below a critical energy level (Heimpel and Collier 1996).

Dynamic state-variable models include several physiological and ecological factors that affect the host-feeding behaviour. Among them are host quality, host abundance, host distribution, egg load, gut contents/nutrient reserves, parasitoid age, mortality risk, egg maturation delay, egg resorption cost and inability to resorb eggs. The dynamic state-variable models predict that host-feeding should increase when female’s egg load is low, when there is oosorption, when mortality risk is low, when nutrients reserves and gut contents are low, and when host quality is low. Models also predict that host-feeding should decrease at older age (Heimpel and Collier 1996).

3. Parasitoids

Definition

A parasitoid is "an organism which develops on or in another single organism, extracts nourishment from it, and kills it as a direct or indirect result of that development" (Eggleton and Gaston 1990). Parasites and parasitoids are different. Unlike parasites, parasitoids use only one host and their development always leads to the dead of the host. The term “parasitoid” is usually applied to insect taxa that exhibit this particular parasitic way of life but parasitoids are also found in taxa such as nematodes, fungi, protista and virus (Godfray 1994).

Description

A typical parasitoids' life cycle is divided into four stages (egg, larva, pupa and adult). Parasitoids can attack all host stages and thus express diversity in host's exploitation. Depending if they develop inside or outside their hosts, they can be classified as endo- or ecto-parasitoids. When they allow the host to continue to grow after parasitism and kill it at the end of their development, they are called koinobionts while when the host is killed or paralized at oviposition, they are called idiobionts (Askew and Shaw 1986). They can also be solitary, when only one parasitoid can achieve its development per host, or gregarious, when more than one parasitoid can develop in a host. Solitary parasitoid species that attack aggregated hosts are called quasi-gregarious (van den Assem et al. 1980). Hymenopteran parasitoids can reproduce by arrhenotokous parthenogenesis or by thelytokous parthenogenesis. In the first mode, females come from fertilized eggs and males from unfertilized eggs and in the second one, only females are produced in absence of mating (Godfray 1994).

There are around 100 000 described species of parasitoids found in seven orders. The greatest number is found in the order Hymenoptera, which accounts for nearly 75% of the estimated number of parasitoid species (Feener and Brown 1997, Belshaw et al. 2003, Santos and Quicke 2011). Second arrive the order Diptera (22%) and then we find fewer species in Coleoptera, Lepidoptera, Trichoptera, Neuroptera and Streptisera (Quicke 1997).

Parasitoids are of great importance in biological control programs and are interesting insects to study because of their unique lifestyle and their particular relation with their host. They were chosen as models in this study especially for their scientific and economic interest.

3.1 Egg parasitoids

Definition and Description

An egg parasitoid is a parasitoid that attacks and develops into the egg stage of its host. Egg parasitoids are among the smallest insects and in fact, one of the smallest insect known is an egg parasitoid of Psocoptera measuring about 140 µm: Mymaridae *Dicopomorpha echmepterygis* Mockford (Mockford 1997). They attack and complete their development into the eggs of several insect species including insect pests, which make them good biological control agents. Their small size also implies that throughout their life, egg parasitoids face several constraints. Their lifetime is very short, within the range of days or even hours and their reserve of energy is at best limited. Any meaningful movements within the habitat are also problematic for them (Boivin 2010). Egg parasitoids are defined on their lifestyle but come from different Hymenopteran families: Trichogrammatidae, Mymaridae, Eulophidae, Scelionidae, Aphelinidae and Encyrtidae (Boivin 2010).

Trichogrammatidae

The Trichogrammatidae family is part of the hymenopterous Chalcidoidea superfamily and is divided into two subfamilies: Trichogrammatinae and Oligositinae (Pinto 2006, Owen et al. 2007). We find 89 genera and more than 800 species of this family distributed in the six biogeographical regions (Pinto and Stouthamer 1994, Querino et al. 2010). They are all arrhenotokous or thelytokous parasitoids. Species of Trichogrammatidae are solitary or facultative gregarious endoparasitoids of insect eggs. Several orders of insects are attacked by Trichogrammatidae such as Hemiptera, Coleoptera, Lepidoptera, Diptera, Orthoptera, Odonata, Hymenoptera, Neuroptera and Thysanoptera (Querino et al. 2010).

Trichogramma

Trichogramma genus is the largest and best known of the Trichogrammatidae family with its 210 species (Pinto 2006, Querino et al. 2010). *Trichogramma* species have been found in all terrestrial habitats sampled. The fact that more species are described in this genus is certainly linked to their importance in biological control programs. Two species of T*richogramma* were chosen for this study because of their ability of controlling pests and because of their easiness of rearing. The fact that they are egg parasitoids and they face several constraints such as they are limited by the amount of resource their host contains, also determined the choice of these two species.

a) *Trichogramma brassicae* Bezdenko

Trichogramma brassicae Bezdenko originates from Moldavia (Black Sea region) and has been introduced into several countries in Central Europe and in North America. Several crop pests such as the rice stem borer *Chilo suppressalis* Walker, the European corn borer, *Ostrinia nubilalis* Hübner, the cotton bollworm *Helicoverpa armigera*

Hübner, and some related species are target hosts of *T. brassicae* (Li 1994, Dutton et al. 1996, Iranipour et al. 2009).

b) *Trichogramma euproctidis* Girault

The *Trichogramma euproctidis* Girault strain used in this study originates from Egypt but that species is found in Europe, Asia and Africa (Thompson 1958). The species attacks several lepidopteran eggs (Quicke 1997).

3.2 Reproductive strategies in *Trichogramma*

Sex allocation and progeny allocation

When a female parasitoid encounters a suitable host, it has to take two decisions in her reproductive strategy: what sex ratio to produce and how many eggs to lay (Waage and Ming 1984). *Trichogramma* wasps are haplodiploid insects in which the sex ratio varies from slightly male-biased to all females. These variations are under female control since they can regulate egg fertilization. Males are haploid and come from unfertilized eggs while females are diploid and come from fertilized eggs. Females *Trichogramma* are moderately synovigenic (Ferracini et al. 2006) which mean that they emerge with a certain number of mature eggs, but have the capacity of producing more during their life. Females lay the majority of their eggs shortly after emergence (Fleury and Boulétreau 1993) and the maturation of new eggs increases by 70 % after a host-feeding meal (Ferracini et al. 2006). Females may lay one or more eggs (clutch size) per host and progeny allocation depends on several factors such as the time needed for laying an egg, rate of host finding, mortality risk, fitness realized by egg, egg load, female experience, female age and host quality and density (Waage 1986). The host egg has a limited amount of food for progeny and thus, the female decisions are of great importance. This amount of resource will determine its progeny survival and fitness once adults. More immatures per host also mean more competition for resource and less resource per individuals. The number of immatures developing per host will therefore has an impact on adult size and size influences fitness through fecundity and longevity (Waage and Ming 1984). *Trichogramma* use what we call «precise sex allocation» (Luck et al. 2001). A male is generally deposited first in a sequence of host encountered and another male may be deposited later as the number of encounters increases (Godfray 1994). The early production of males is a good strategy for these minute egg parasitoids that are unable to assess the size of the egg mass they are parasitizing and it ensures that they never run out of host eggs to parasitize before they have laid at least one son (Godfray 1994). The other son deposited later in the sequence ensures that there is just enough son to fertilize all the deposited daughters as the number of encounters increases.

4. Host

Trichogramma species can attack more than one order of hosts or be specialized on one order (Querino et al. 2010). *Trichogramma euproctidis* is known to attack Diptera and Neuroptera, but is mostly specialized on lepidopteran eggs (Polaszek 2010). Major lepidopteran crop pests such as *Chilo suppressalis*, *Ostrinia nubilalis* and *Helicoverpa armigera* are attacked by *Trichogramma brassicae* (Li 1994, Dutton et al. 1996, Iranipour et al. 2009) but it can also attack dipteran species such as *Lydella thompsoni* Herting (Polaszek 2010). *Trichogramma* spp. can be easily reared on laboratory hosts such as *Ephestia kuehniella* Zeller, *Sitotroga cerealella* Olivier, *Plodia interpunctella*

Hübner, *Galleria mellonella* Linnaeus and *Corcyra cephalonica* Stainton (Iranipour et al. 2009).

***Ephestia kuheniella* Zeller**

The Mediterranean flour moth, *Ephestia kuehniella*, is a lepidopterous pest from the Pyralidae family, commonly found in stored grain and flour (Richardson 1926). It is the commonest *Ephestia* found in North America. This species is largely used for parasitoid mass rearing because of its resistance against diseases and its ease to rear (Richardson 1926). The egg volume usually allows for the development of a single *Trichogramma* (Bai et al. 1992). The eggs are beige, round (0.5x 0.3 x 0.3 mm) and have a volume of approximately 0.28 mm^3 (Bai et al. 1992).

5. Biological control with *Trichogramma*

Biological control is defined as the "regulation by natural enemies of another organism's population density at a lower average density that would otherwise occur" (DeBach 1974). To prevent pest population from reaching the injury level, we usually use inoculative biological control, where a species is introduced early in the season to establish itself and to protect crops (Li 1994). In contrast, inundative biological control is used for an immediate effect on pests and a large number of individuals are released (Li 1994). In classical biological control, the importation and establishment of exotic natural enemies is used to add a density-dependent mortality factor for a specific pest, generally exotic too (DeBach 1974).

The first attempt on using *Trichogramma* for biological control was undertaken in 1900 in the USA with two exotic species from Austria (Luck and Forster 2003). Now, over 32 million ha are treated every year with *Trichogramma* species for the biocontrol of over 20 host-pests, generally lepidopterous pests, on corn, sugar cane, cotton, fruit trees and vegetables (Li 1994, Smith 1996, Parra 2010). More than 30 countries use *Trichogramma* and this number increases continuously (Li 1994, Parra 2010). Among the principal countries we find Russia, China and Mexico with over two million ha treated every year (Li 1994, Parra 2010) and a notable increase in establishment of biofactories in Latin America has been observed in the last years (Parra 2010).

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CHAPTER II

Effect of low temperature on emergence, fecundity, longevity and host-feeding by *Trichogramma brassicae*

Émilie Lessard & Guy Boivin

Abstract:

The occurrence of sub-optimal temperatures during development of immature parasitoids can have important consequences on adult fitness. We investigated the impact of different regimes of low temperature on emergence, differential mortality, longevity and fecundity in *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae). The host-feeding behaviour of adult females was also measured as an indicator of energy reserve at emergence. Acclimation of 30 days at 10°C or 24 days at 13°C allowed *T. brassicae* immatures to develop with a lower mortality than those without an acclimation period when all larvae were exposed directly at 5°C. Longevity and fecundity of females decreased at a lower rate with acclimation at 10°C suggesting that acclimation at 13°C may have depleted the energy reserves of individuals more than acclimation at 10°C. Short photoperiod exposure during the maternal generation had no effect on progeny's fitness. We found no difference among the treatments in females' host-feeding behaviours, in differential mortality at emergence, in female's mobility and in F1 sex ratio.

Introduction:

Development under sub-optimal temperature can have important consequences on the fitness of the resulting adult insect. These impacts are of interest in parasitoid insects, as their efficiency when used in biological control programs can be seriously affected. Low temperature can cause physiological dysfunctions (Colinet and Boivin 2011), depletion of energy reserves (Chen et al. 2008) and morphological alterations such as malformations of reproductive organs (Denlinger and Lee 1998), a reduction in body size (Rundle et al. 2004), wings deformity (Dutton and Bigler 1995; Tezze and Botto 2004) and alteration of antennal structure (Pintureau and Daumal 1995). Authors have reported a reduction in percentage of emergence (Uçkan and Gülel 2001; Bayram et al. 2005; Özder and Saglam 2005), a decrease in longevity (Jalali and Singh 1992; Pandey and Johnson 2005) and fecundity (Levie et al. 2005; Pandey and Johnson 2005), a drift in emerging sex ratio towards more males (Bayram et al 2005; Chen et al. 2008) and a reduction in parasitoid's mobility (Tezze and Botto 2004; Ayvaz et al. 2008).

At low temperature, even if metabolism slows, development of immatures takes longer and more resources have to be allocated for metabolic maintenance at the detriment of other traits such as reproduction (Boivin 2010). In the case of idiobiont parasitoids, the resources available to the developing immature are fixed as the host is killed or paralysed at the time of oviposition (Godfray 1994). Thus, idiobiont parasitoids such as egg parasitoids are more constrained when subjected to low temperature as immatures than koinobiont, where the parasitoid allows hosts to continue to grow in size after parasitism (Godfray 1994).

The negative consequences of low temperature during immature development may also affect the behaviours associated with foraging for hosts. For instance, low temperature of 4°C during prepupal stage affects the locomotor activity in *Trichogramma nerudai* Pintureau and Gerding, resulting in a lower ability to parasitize (Tezze and Botto 2004). Behaviours associated with host location and evaluation can also be modified by reducing the parasitoid's learning and discrimination capacities (Hance et al. 2007). When the immatures of the mymarid *Anaphes victus* Huber (Hymenoptera: Mymaridae) are exposed to 4°C for 3 to 12 weeks, the resulting adults have reduced capacities of learning external oviposition marks and also to optimize their patch time allocation (van Baaren et al. 2005). Low temperature can affect negatively the fat reserves of the emerging adult (Chen et al. 2008) and individuals with a lower energy reserve may have to search for food sources instead of concentrating their effort on reproduction (Lewis et al. 1998). If the energy reserves and budget of adults are modified by the conditions experienced during their immature development, behaviours such as host-feeding could reflect these changes.

Host-feeding, defined as the consumption of host heamolymph and tissues by the adult females (Jervis and Kidd 1986), could increase with an increase in the costs associated with low temperature exposure. The existing models for host-feeding predict that females host-feed more frequently when their egg load is low and when their nutrients reserve is low (Heimpel and Collier 1996). Observation of the host-feeding behaviour could allow us to measure indirectly the impact of low temperature on the energy reserve of the adult. The negative consequences on fitness are generally proportional to both the temperature and the duration of exposure (Colinet and Boivin 2011) and we expect that host-feeding frequency or intensity should also be proportional.

In nature, gradual changes in seasons allow the parasitoid insect to prepare for the cold season. We refer to "acclimation" when gradual physiological response to altered environmental conditions occurs in laboratory and "acclimatization" when it occurs in nature (Withers 1992; Levie et al. 2005). In addition to the decreasing temperature, the reduced photoperiod is another cue used to prepare for the incoming cold period in nature, but its effect is often only slight over one generation in *Trichogramma* species (Boivin 1994; Laing and Corrigan 1995; Ivanov and Reznik 2008). The immatures of several *Trichogramma* species can enter diapause or become quiescent within their host eggs, allowing them to tolerate long periods of suboptimal temperature (Pitcher et al. 2002).

In this paper, we measured the impact of low temperature during immature development on *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) fitness (percentage of emergence, fecundity of adult, longevity of adult, F1 sex ratio and time taken to parasitize ten different eggs (used as an indicator of adult's mobility)) with different acclimation regimes and with short photoperiod experienced by the maternal generation in laboratory conditions. We also determined if acclimation modified the females' host feeding behaviour compared to direct low temperature exposure without acclimation.

Material and methods

Insect rearing

The two *Trichogramma brassicae* strains (named B5 and B6) used in those experiments are commercially available in Canada (Anatis Bioprotection Inc.). Eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) were from a laboratory culture. *Trichogramma brassicae* species originated from Moldavia (Black Sea region) and has been introduced into several countries in Central Europe and in North America, mostly to control the European corn borer (*Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae). The parasitoids were reared on cold-killed *E. kuehniella* eggs at 25 ±2°C, 50±5% r.h. and photoperiod of L16:D8. The developmental threshold for this species is approximatively 10°C (Babendreier et al. 2003; Rundle and Hoffmann 2003; Rundle et al. 2004) and no development occurs at 5°C (Rundle and Hoffmann 2003) in *Trichogramma* spp.

Experiment 1

60 ± 30 newly emerged and mated females of each *T. brassicae* strain were allowed to parasitize 0.1±0.02g of *E. kuehniella* eggs (around 4000 eggs) for 24h in a petri dish. This ratio females/host eggs ensured a good percentage of parasitism and low superparasitism (trials were done previously). Eggs from the exposure petri dish were then placed on small strips of 3 M Post-it® (200±50 eggs per strip) (Leatemia et al. 1995; Martel et al. 2011) that were placed in glass vials (3.7 ml) with a muslin cap for aeration. The glass vials were then subjected to five different treatments (including control) that were repeated five times.

Control: For each replicate, one vial of each strain was placed at 25 ±2°C, 50±5% r.h. and photoperiod of L16:D8 until emergence of adults.

No acclimation: to assess the impact of a direct transfer to low temperature without acclimation, for each replicate, four vials per strain were placed directly at 5 ±1°C, 30±10% r.h., in complete darkness for 4, 8, 12 and 16 weeks.

Acclimation at 13°C: to evaluate the impact of acclimation at low temperature, for each replicate, five vials per strain were placed at 13 ±1°C, 55±10% r.h and L12: D12 for 24 days prior to being transferred at 5 ±1°C, 30±10% r.h., in complete darkness for 0, 4, 8, 12 and 16 weeks.

Acclimation at 10°C: to assess the impact of acclimation to a temperature closer to the development threshold of 10°C for *Trichogramma brassicae* (Babendreier et al. 2003; Rundle and Hoffmann 2003; Rundle et al. 2004), for each replicate, five vials per strain were placed in an incubator maintained at 10 ±1°C, 60±15% r.h and L12: D12 for 30 days prior to being transferred at 5 ±1°C, 30±10% r.h., in complete darkness for 0, 4, 8, 12 and 16 weeks.

Acclimation at 13°C and short photoperiod: to evaluate the impact of short photoperiod during the maternal generation, for each replicate, one vial per strain was placed in an incubator at 25 ±2°C, 50±5% r.h. and L10 :D14 until emergence of adults. These females were allowed access to a petri dish containing 0.1±0.02g of *E. kuehniella* eggs during 24 hours. The exposed period was also held under the short photoperiod conditions. We used 60 ± 30 newly emerged and mated females for the exposure. Parasitized eggs were placed on small strips made of 3 M Post-it® (200±50 eggs per strip) and placed in vials that experienced the same conditions described in "Acclimation at 13°C" treatment.

After each treatment, vials were brought back to 25°C, 50±5% r.h., L16:D8 until emergence that was checked daily. On the emergence day, five females per vial were isolated to assess 48h-fecundity and longevity. To assess 48h-fecundity, each isolated female received cold-killed eggs of *E. kuehniella* glued on a Post-it® strip (200±50 eggs per strip). After 48 hours, the strip was removed and incubated for six days after which parasitized eggs that had turned black were counted to determine fecundity. To assess longevity, the isolated females were observed daily until death. Death was assumed to occur at mid-point between the last two observations. Neither water nor food was offered to the females but a new strip with cold-killed eggs of *E. kuehniella* was provided to the females until death.

In order to assess the percentages of emergence, the total number of adults emerged was divided by the total number of hosts that turned black during the experiment. The differential mortality between sexes was also measured by comparing the weekly sex ratio within a treatment. Vials were verified for emergence until no emergence occurred for 8 weeks.

Experiment 2

The impact of low temperature during immature development on the frequency and intensity of host-feeding by adult females was evaluated through behavioural observations. Parasitized eggs of strain B6 were subjected to three treatments. The parasitized eggs were glued on Post-it® paper strips (200±50 eggs per strip) after contacts and then placed in vials as in experiment 1. In the first and second treatments, the vials were kept for 30 days at 10 ±1°C, 60±15% r.h and L12: D12 and, for second treatment only, the vials were in addition subjected to 4 weeks at 5 ±1°C, 30±10% r.h. and in complete darkness. All the vials were then brought back to 25±2°C, 50±5% r.h., L16:D8 until emergence. For the last treatment (control), the vials were kept at 25 ±2°C, 50±5% r.h. and photoperiod of L16:D8 until emergence. A total of 66 females (22 per treatment) were observed in the 10 hours following their emergence. Neither water nor food was offered to the females prior to the observations.

Trichogramma brassicae females were offered a square patch of 16 fresh (less than 24 hours) *E. kuehniella* eggs on a filter paper of 12.5 mm under laboratory conditions (25±2°C, 30±5% r.h.). The distance between each egg was 3.0 mm. The behaviours observed were oviposition, walking, resting and host-feeding. The behaviours were recorded using The Observer XT software, a camera and a binocular microscope. Observations started when the female parasitized its first host and ended after 10 different hosts were parasitized. Time taken to parasitize 10 different hosts was used as an indicator of females' mobility. The few females (Control: 2, "Acclimation at 10°C": 3, "Acclimation at 10°C + 4 weeks at 5°C" : 4) that did not reach ten eggs were excluded from statistics.

At the end of each observation, parasitized eggs were transferred into Beem® polyethylene capsules and incubated at 25 ±2°C, 50±5% r.h. and photoperiod of L16:D8 until emergence. Progeny wasps were then sexed and sex ratio was calculated.

Statistical analyses

Statistical analyses were done using R 2.11.1 software. Data did not conform normal distribution (Shapiro-Wilk normality test) and non-parametric statistics were chosen. In experiment 1, comparisons between weeks within a treatment and within a strain were done using Kruskal-Wallis tests and, if significant at $P<0.05$, followed by a non-parametric multiple comparison test. Mann-Whitney tests have been used to compare ”Acclimation at 13°C” and ”Acclimation at 13°C and short photoperiod” treatments for the maternal effect and to compare both strains. In experiment 2, comparisons between the three treatments were done using a Kruskal-Wallis test.

Results:

Experiment 1

In general, strains B5 and B6 responded the same way to the treatments and only few significant differences were observed between those strains (8 significant results out of 53 tests). The few significant results showed no pattern. For that reason, both strains were combined for analysis. The median percentages of emergence for the control was 88.4% (Q1 = 84.0%, Q3 = 97.1%). We observed emergence up to 16 weeks at 5 ±1°C in “Acclimation at 13°C”, “Acclimation at 10°C” and “Acclimation at 13°C and short photoperiod” treatments (Figure 2.1 B, C, D) while no emergence was observed after 12 weeks in the “No acclimation” treatment (Figure 2.1 A). Since 16 weeks is the longest duration at low temperature tested in this experiment and there was still emergence after this period with acclimation, we kept 16 weeks as a reference to compare acclimation treatments between them (Table 2.1). Note that in all figures, the control is the same and in the "No acclimation" treatment, it also corresponds to 0 week at 5°C. In the other treatments, the 0 week corresponds to no storage at 5°C following the acclimation. The Kruskal-Wallis tests between weeks within a treatment including control showed few differences in differential mortality between sexes (No acclimation: W= 7.289, df = 2, P = 0.026; Acclimation at 13°C: W = 8.845, df = 5,P = 0.115; Acclimation at 10°C: W= 11.227, df = 5 ,P = 0.471; Acclimation at 13°C and short photoperiod: W = 2.749, df = 5, P= 0.739).

Longevity decreased with duration of exposure at 5°C with the acclimation temperature of 13°C (Figure 2.2B), but stayed constant when the acclimation temperature was 10°C (Figure 2.2C). Only two females emerged in the "No acclimation" treatment after 8 weeks (Figure 2.2A) and one of these females did not survive the first 48 hours. Surprisingly, the maximum longevity was observed in the “No acclimation” treatment after 4 weeks spent at 5 ±1°C (Median = 4.5 days, Q1 = 2.5 days, Q3 = 4.5 days) and not in the control (Median = 2.5 days, Q1 = 1.5 days, Q3 = 3.5 days). Longevity was also higher in the “Acclimation at 10°C” treatment after 16 weeks compared with “Acclimation at 13°C” and “Acclimation at 13°C and short photoperiod” (Table 2.2).

Fecundity decreased gradually with duration of exposure at 5°C but acclimation at 10°C and 13°C maintained it higher for a longer period (Figure 2.3 B, C, D). Fecundity after 16 weeks in the “Acclimation at 10°C” treatment was superior to fecundity after 16 weeks in the “Acclimation at 13°C and short photoperiod” treatment (Table 2.2). Comparison after 16 weeks with the other treatments was not possible since females did not survive enough longer to ensure good fecundity tests. Females of control treatment successfully parasitized 56 eggs (Q1 = 52 eggs, Q3 = 60 eggs) in 48 h (Figure 2.3).

The comparisons between “Acclimation at 13°C” (without maternal effect) and “Acclimation at 13°C and short photoperiod” (with maternal effect) showed that placing the previous generation at short photoperiod had little effect on the parameters measured (Table 2.1). Only the median fecundity after 4 weeks ($p = 0.019$) was significantly higher for “Acclimation at 13°C and short photoperiod”.

The host eggs turned black after 24 days at 13°C and 30 days at 10°C which is a sign that development occurred at 13°C and 10°C and the prepupal stage was reached after both acclimation treatments.

In all treatments, when the vials were brought back to 25 ±2°C, 50±5% r.h. and photoperiod of L16:D8, a single peak of emergence was observed and no emergence was observed later, even after 8 weeks, suggesting that these insects were in a quiescent state during the treatment rather than in diapause.

Experiment 2

The time taken to parasitize 10 hosts (the female’s mobility), the frequency (number of host-feeding bouts during the observations) and duration (time spent doing host-feeding during the observations) of host-feeding were not different between “Acclimation at 10°C” treatment, “Acclimation at 10°C treatment + 4 weeks at 5°C” and the control (Table 2.3). The number of eggs used for host-feeding during the observations and the sex-ratio obtained in F1 generation were also similar between the three treatments. Only data from females performing host-feeding were kept for comparisons (zeros were excluded of the calculation after a chi-square test (5/22, 3/22, 6/22), df = 2, Chi2 =0.843, p-value = 0.530).

Discussion :

Even at non-freezing temperature, immature parasitoids that develop at sub-optimal temperature usually suffer major fitness costs (van Baaren et al. 2005; Hance et al. 2007; Colinet and Boivin 2011). However, preconditioning individuals by exposing them to low temperature close to their developmental threshold can improve parasitoid survival (Pandey and Johnson 2005). Our results show that *T. brassicae* can survive and emerge after up to 16 weeks at 5°C with an acclimation period of 24 days at 13°C or 30 days at 10°C. Without acclimation, the majority of individuals did not emerge after only four weeks at 5 °C and 100% did not emerge after 12 weeks. Özder and Saglam (2005) observed similar results with *T. brassicae* and a significant decline in emergence was observed after three weeks at 4°C. A gradual decrease in longevity and in fecundity is generally observed in egg parasitoids as the duration of low temperature exposure increases (Colinet and Boivin 2011). Acclimations at 10°C and 13°C both improved the parasitoids' fitness, but 30 days at 10°C was the best treatment to acclimate *T. brassicae*. When we compare the acclimation treatments after 16 weeks spent at 5°C, females subjected to an acclimation treatment at 10°C obtained higher longevity and fecundity. The relative humidity during storage (30% r.h.) was probably too low for optimal storage of *T. brassicae* and could account for some of the mortality observed. However, acclimation at higher temperature decreased the level of mortality suggesting that low relative humidity was not the major mortality factor.

The duration of exposure and acclimation temperature are both very important. During the acclimation of 30 days at 10°C, and even 24 days at 13°C, the immature continued to develop and reached the prepupal stage by the end of this period since the host eggs had turned black. The prepupal stage is less sensitive to low temperature than the larvae in *Trichogramma* spp. (Garcia et al. 2002). The better results obtained after acclimation at 10°C rather than 13°C or rather than 13°C and short photoperiod could be explained by the fact that the metabolic rate was lower at 10°C and therefore that individuals acclimated at 10°C entered storage at 5°C with higher energy reserve than individuals acclimated at 13°C. It could also explain the higher longevity and fecundity obtained after 16 weeks when acclimation at 10°C was used. The fact that 10°C appear to be the approximate development threshold for *T. brassicae* (Babendreier et al. 2003; Rundle and Hoffmann 2003; Rundle et al. 2004) support the hypothesis of a low metabolic rate at 10°C.

Acclimation can also have a cost and its global effect on fitness will depend on the overall balance between costs and benefits (Hoffmann and Hewa-Kapuge 2000; Rako and Hoffmann 2006). Here, the cost of being at suboptimal temperature included a decrease in percentage of emergence, longevity and fecundity, but the cost did not overcome the benefits of acclimation. Our results are similar to what has been described in other *Trichogramma* species. *Trichogramma ostriniae* Pang and Chen can be stored at 9 or 12°C for 4 weeks without a decline in percentage of emergence, longevity and fecundity (Pitcher et al. 2002) and the same range of temperatures is acceptable to store *Trichogramma evanescens* Westwood (Iacob and Iacob 1972 cited in Pitcher et al. 2002 and Jalali and Singh 1992). *Trichogramma achaeae* Nagaraja and Nagarkatti*, T. eldanae* Viggiani*, T. chilonis* Ishii and *T. japonicum* Ashmead see their fecundity and longevity decline drastically after 21 days at 10°C (Jalali and Singh 1992) and are thus a little more sensitive than our species. Our results showed that *T. brassicae* can be exposed to an additional 4 weeks at 5°C after the acclimation period without much decrease in fitness. Acclimation can therefore protect *Trichogramma*'s fitness and quality and should be considered in storage protocols.

When exposed to low temperatures, insects may enter in two types of dormancy in order to survive adverse conditions: quiescence and diapause (Danks 1987). Quiescence is an immediate response to a limiting factor but resumption of development occurs as soon as the conditions improve while diapause is a more profound interruption of development, a programmed response usually preceding the adverse conditions and that lasts longer than the adverse conditions (Danks 1987). *Trichogramma* spp. can use diapause and quiescence against low temperature (Boivin 1994). The fact that a single peak of emergence was observed shortly after the vials were brought back to 25°C, no matter how many weeks they have spent at 5°C, suggests that *T. brassicae* was in a quiescent state in all treatments. An immediate resumption of development when adverse conditions end is typical to quiescence (Chen et al. 2008). Diapause of *T. brassicae* in natural conditions can last 132 days (Özder and Saglam 2005b) but we exceeded that period (acclimation period + X weeks at 5°C + 8 weeks of watching at 25°C) without seeing other emergences from the black host eggs remaining after the first peak. In many cases, the remaining black eggs were in bad conditions and dry. The two main factors known to induce *Trichogramma* spp. dormancy are the photoperiod, especially on the maternal generation, and the temperature (degree and time of exposure) (Zaslavski and Umarova 1990; Boivin 1994; Ivanov and Reznik 2008; Pizzol and Pintureau 2008). Conditions similar to the ones we used (30 days at 10°C and 24 days at 13°C) induce diapause in other species of *Trichogramma* (Pintureau and Daumal 1995; Garcia et al. 2002; Ma and Chen 2006), but our two strains only entered quiescence.

Although a short photoperiod during the maternal generation is known to influence positively diapause induction in *T. evanescens, T. embryophagum* Hartig*, T. aurosum* Sugonjaev and Sorokina*, T. pintoi* Voegele*,* and *T. cacoeciae* Marchal (Zaslasvki and Umarova 1990; Smith 1996; Ivanov and Reznik 2008; Pizzol and Pintureau 2008; Reznik et al. 2008), it did not induce it in *T. brassicae*. The short photoperiod experienced by the mother could have however prepared its progeny to low temperature exposure by reducing the fitness cost associated with it but our results showed that there is no positive maternal effect of photoperiod in quiescent *T. brassicae* either.

Not all fitness parameters measured were affected by exposure at low temperature. Both sexes had similar survival with the temperatures and duration of exposure tested here. Neutral effect on differential mortality was also observed in *T. pretiosym* Riley*, T. atopovirilia* Oatman and Platner*, T. acacioi* Brun, Moraes and Soares*, T. rojasi* Nagaraja and Nagakatti*, T. lasallei* Pinto and *T. evanescens*. (Ayvaz et al. 2008; Foerster and Foerster 2009).

Female parasitoids use host-feeding to obtain resources and the intensity of host-feeding should reflect the status of their reserves (Heimpel and Collier 1996). We hypothesized that females that experienced longer duration of sub-optimal temperature during their immature development could have lower energy reserve as they emerge and therefore express more and longer host-feeding bouts. Surprisingly, *T. brassicae* females did not increase their host-feeding behaviour after storage at 10°C and exposure at 5°C during 4 weeks compared with control treatment. During development, the accumulated reserves are allocated between somatic and reproduction functions (Colinet et al. 2007). Immature females placed below 10°C (below developmental threshold) must still use some resources for somatic maintenance (Rundle et al. 2004) and burn their fat body reserves (Colinet et al. 2007; Chen et al. 2008) in order to survive adverse conditions. This results in fewer resources for reproduction (Colinet et al. 2007). Particularly for small parasitoids, fat reserves are a non-replaceable resource and potentially a limiting factor (Rivero and West 2002). *Trichogramma* females can gain proteins, amino acids and lipids by host-feeding on Lepidoptera eggs and as they are moderately synovigenic (i.e. they emerge with part of their egg complement but they are able to produce more during their life (Jervis et al. 2001)), those nutrients are used both for sustaining egg production and for somatic maintenance (Ferracini et al. 2006). The fact that females did not increase their host-feeding behaviour suggests that the fitness cost associated with low temperature exposure was not important enough to modify the frequency or intensity of host-feeding.

The sex ratio of the F1 generation in the second experiment was not different from the control and time taken to parasitize 10 different eggs was the same among treatments. This indicates that low temperature did not affect the fertility of male or the capacity of females to move and localize hosts. In *T. evanescens*, a decrease in the percentage of females in F1 generation after low temperature exposure of parental lines has already been observed (Abd El-Gawad et al. 2010). Some studies have reported alteration in parasitoid's mobility caused by low temperature injuries when no acclimation was used. For instance, when *T. evanescens* immatures are exposed to 4°C for 28 days, the resulting adults see their walking speed decrease (Ayvaz et al. 2008) and when *T. nerudai* immatures are exposed at the same temperature for 50 days, the flying ability of the resulting adults is affected negatively (Tezze and Botto 2004).

The fitness cost associated with low temperature exposure is reduced in *T. brassicae* when acclimation is used and 10°C during 30 days gave better results than 24 days at 13°C. Both treatments allowed *T. brassicae* to reach the prepupal stage but acclimation at 13°C may have depleted the energy reserves of individuals more than acclimation at 10°C. Acclimation should therefore be used when *Trichogramma* immatures are stored for a long period in biological control programs.

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Table 2.1 Comparisons between “Acclimation at 13°C” (without maternal effect) and “Acclimation at 13°C and short photoperiod” (with maternal effect) for percentage of emergence, longevity and fecundity of *T. brassicae* females

	Percentage of emergence		Longevity		Fecundity	
	W	p-value	W	p-value	W	p-value
0 week	32.5	0.205	630.0	0.289	353.5	0.776
4 weeks	73.0	0.089	1198.5	0.708	20.5	0.019*
8 weeks	73.0	0.089	1205.0	0.793	0.0	0.333
12 weeks	65.0	0.272	704.5	0.065	NA	NA
16 weeks	40.5	0.744	446.5	0.932	NA	NA

* $P<0.05$; Mann-Whitney test, NA means “Not Available”

Table 2.2 Comparisons between the four treatments for percentage of emergence, longevity and fecundity after 16 weeks at 5°C. Different letters indicate significant differences at $P<0.05$ between treatments (after a multiple comparison test following a Kruskal-Wallis test). NA means that date were not available since no emergence occurred or females did not live 48h.

		No Acclimation	Acclimation at 13°C	Acclimation at 10°C	Acclimation at 13°C and short photoperiod
% of emergence	Median	NA	21^a	32^a	21^a
Longevity	Median	NA	0.5^b	3.0^a	0.5^b
Fecundity	Median	NA	NA	29^a	23^b

Table 2.3 Effect of each treatment on time taken to parasitize 10 different hosts, on host-feeding frequency, on host-feeding duration, on the number of hosts used for host-feeding and on sex ratio in F1 generation with *T. brassicae* B6 strain. The medians (M) and quartiles (Q1, Q3) are shown for each treatment.

	Acclimation at 10°C			Acclimation at 10°C + 4 weeks at 5°C			Control			Kruskal-Wallis test*	
	M	Q1	Q3	M	Q1	Q3	M	Q1	Q3	W	p-value
Time taken to parasitize 10 different hosts (sec)	2090.0	1721.0	2617.0	2106.5	1900.3	2571.8	2054.0	1647.0	2413.0	0.1982	0.906
Frequency of Host-Feeding	2.0	1.0	3.0	2.0	1.0	4.3	1.0	1.0	2.0	2.8732	0.238
Duration of Host-Feeding (sec)	98.8	62.4	159.2	89.0	61.2	108.5	51.6	51.6	51.6	3.9789	0.137
Number of hosts used for Host-Feeding	1.0	1.0	2.5	1.0	1.0	2.0	1.0	1.0	1.0	3.4261	0.180
Sex ratio obtained in F1	0.17	0.11	0.25	0.23	0.10	0.33	0.22	0.11	0.30	0.9448	0.624
N		19			16			17			

* df = 2

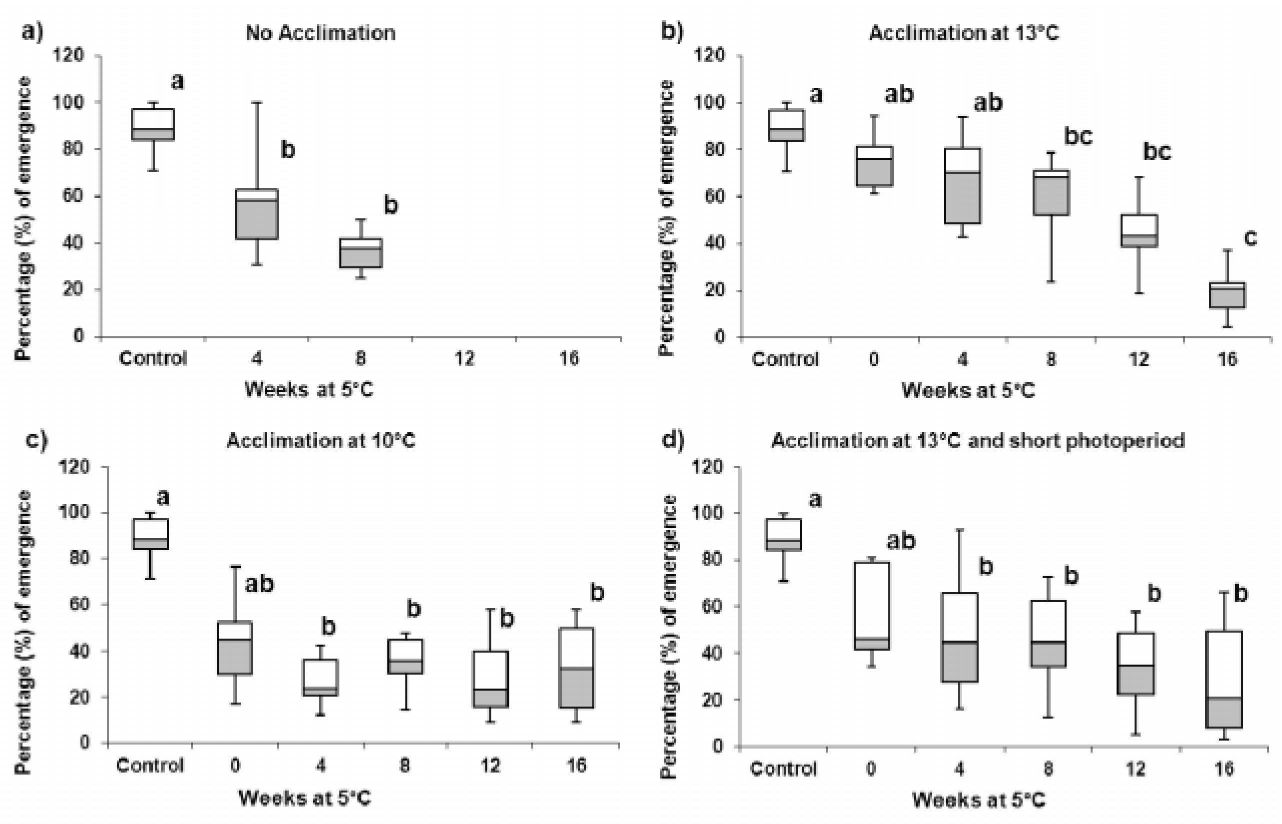

Figure 2.1 Median emergence and quartiles (%) of *T. brassicae* under four treatments. Different letters in a same graph indicate significant differences at P<0.05 between weeks (after a multiple comparison test following a Kruskal-Wallis test)

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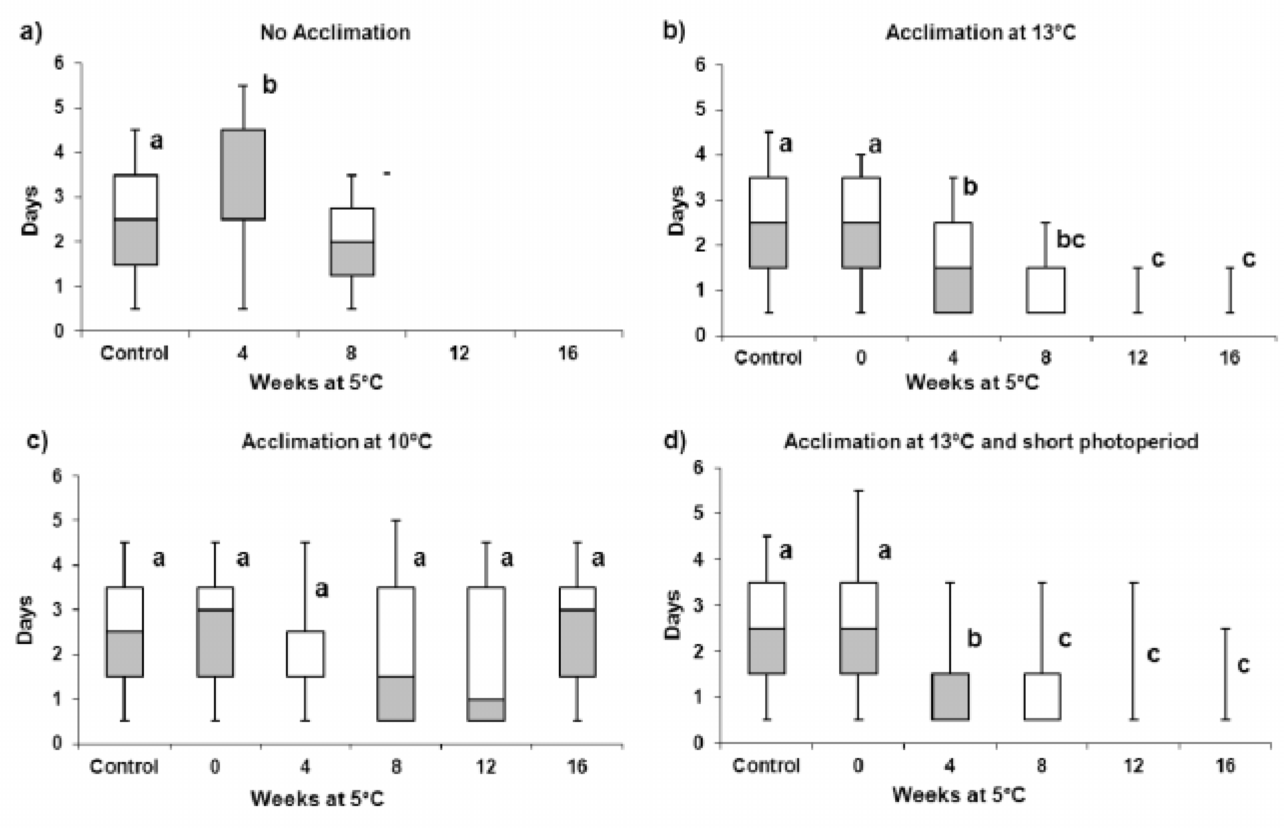

Figure 2.2 Median longevity and quartiles (in days) of *T. brassicae* under four treatments. Different letters in a same graph indicate significant differences at $P<0.05$ between weeks (after a multiple comparison test following a Kruskal-Wallis test). The symbol "-" indicates that two data or less were available

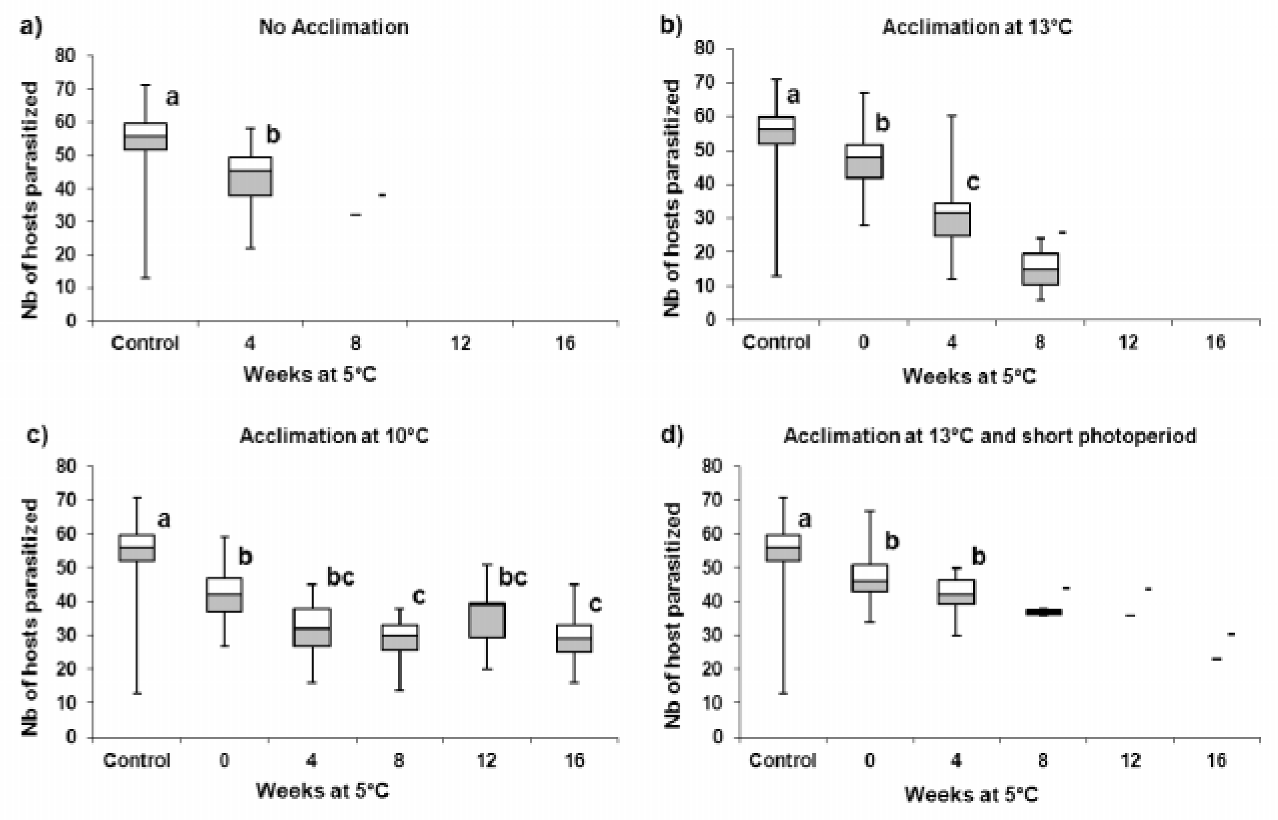

Figure 2.3 Median fecundity and quartiles (in number of hosts parasitized) of *T. brassicae* under four treatments. Different letters in a same graph indicate significant differences at $P<0.05$ between weeks (after a multiple comparison test following a Kruskal-Wallis test). The symbol “-“ indicates that two data or less were available

CONNECTING TEXT

The effects of low temperatures during immature development on several fitness proxies of adults have been discussed in the previous chapter. The decrease in female's energy reserve after low temperature exposure did not result in an increase in host-feeding behaviour contrarily to what we hypothesized. Low temperature during development had a negative impact on emergence, longevity and fecundity but acclimation treatments helped by improving parasitoid survival and by decreasing the fitness costs associated with developing at low temperature. We suggested that the costs associated with low temperature exposure were not important enough to see a difference in energy reserve and in host-feeding behaviour.

Low temperature effects are important to consider since long term storage of insect stocks and synchronization with demands in the field can be achieved by exposing them to low temperature during many weeks or months. However, the behavioural ecology of parasitoids needs further studies concerning host-feeding behaviour and how some important factors such as female's age and hunger level act on the behaviour. In Chapter III, we quantified the effect of age and hunger level of females *T. euproctidis* on the host-feeding behaviour, sex ratio allocated and mobility in a host patch. These results could also help improving mass rearing and biological control with *Trichogramma*.

Chapter submitted to The Canadian Entomologist

CHAPTER III

Effect of age and hunger on host-feeding behaviour by female *Trichogramma euproctidis*

Émilie Lessard & Guy Boivin

Abstract:

Adult parasitoid females can obtain proteins and fat by consuming the hemolymph of their host. In *Trichogramma* spp., host-feeding on the host egg occurs after oviposition and leads to smaller offspring. We tested the effect of age and hunger on host-feeding behaviour of female *Trichogramma euproctidis* Girault (Hymenoptera: Trichogrammatidae). Young and old females, either starved, water-fed or honey-fed, were observed and the host-feeding frequency, duration, distribution and number of hosts used for nutrition were measured. The sex ratio allocated to parasitized hosts where host-feeding occurred and time taken to parasitize 10 hosts (indicator of female's mobility) were also noted. The majority of females host-fed on the first host encountered. Age had no impact on frequency, duration, number of hosts used and mobility of *T. euproctidis*. Starved females host-fed longer and were less mobile. The sex-ratio of the progeny produced on the first host parasitized was more male-biased when host-feeding occurred. Host feeding had no effect on the sex-ratio deposited elsewhere in the sequence. Age of female had no effect on host-feeding, possibly because host-feeding incurs little cost for this species. To host-feed on the first host parasitized, in which a male is allocated, is less costly in term of fitness and represents a strategic choice for the female.

Résumé:

Les femelles parasitoïdes adultes peuvent obtenir des protéines et des lipides en consommant l'hémolymphe de leur hôte. Chez *Trichogramma* spp., la nutrition sur l'œuf hôte se fait après la ponte et cause une réduction de la taille de la progéniture. Nous avons testé l'effet de l'âge et de la faim sur le comportement de nutrition sur l'hôte des femelles *Trichogramma euproctidis* Girault (Hymenoptera : Trichogrammatidae). Des femelles jeunes et vieilles ainsi que des femelles à jeun, nourries à l'eau ou nourries au miel ont été observées et la fréquence, la durée, la distribution de la nutrition sur l'hôte ainsi que le nombre d'hôtes utilisés pour se nourrir ont été mesurés. Le ratio des sexes et le temps pris pour parasiter une séquence de dix hôtes différents (indicateur de la mobilité de la femelle) ont aussi été notés. La majorité des femelles se sont nourries sur le premier hôte rencontré. L'âge n'a pas eu d'impact sur la fréquence, la durée, le nombre d'hôte utilisé pour la nutrition et la mobilité de *T. euproctidis*. Les femelles à jeun se sont nourries plus longtemps et avaient une moins bonne mobilité. La nutrition sur l'hôte était plus fréquente sur le premier œuf hôte rencontré et la proportion de mâles alloués sur cet hôte était plus grande. La nutrition sur l'hôte n'a pas eu d'effet sur le ratio des sexes déposé ailleurs dans la séquence. Contrairement aux prédictions des modèles, l'âge de la femelle n'a pas eu d'effet sur la nutrition sur l'hôte possiblement parce que la nutrition engendre des coûts minimes chez cette espèce. Se nourrir sur le premier hôte parasité, dans lequel un mâle a été alloué, est moins coûteux en terme de valeur adaptative et représente un choix stratégique pour la femelle.

Introduction:

The reproductive success of female parasitoids can be greatly affected by their diet. The importance of finding food sources for somatic maintenance and egg maturation has been well documented (Jervis et al. 1996; Heimpel et al. 1997; Lewis et al. 1998). Female parasitoids gain access to sugars and some proteins by using floral and extrafloral nectar, pollen, as well as honeydew produced by homopteran insects (Jervis et al. 1992). These food sources can be in a different habitat than their hosts and as a result females have to invest time and energy to reach these resources (Jacob and Evans 2001; Bernstein and Jervis 2007; Jervis et al. 2008). Female parasitoids can also obtain proteins and fat by consuming the hemolymph and tissues of their hosts (host-feeding) (Jervis and Kidd 1986). Nutrients obtained from host-feeding appears to be used mainly for egg maturation, although it can be used for somatic maintenance in some species (Jervis and Kidd 1986; van Lenteren et al. 1987; Collier 1995; Heimpel and Collier 1996; Heimpel and Roesenheim 1996; Giron et al. 2004; Ferracini et al. 2006), but it is generally accepted that the role of non-host food is primarily to supply energy for somatic maintenance (Jervis and Kidd 1986; Thompson 1999).

Dynamic state-variable models have been developed to predict how different factors affect a parasitoid's propensity to host-feed. These models balance the risk for the parasitoid of becoming egg- versus time-limited in order to maximize lifetime reproductive success (Heimpel and Collier 1996). They predict that host-feeding should increase when female's egg load is low, when there is oosorption, when mortality risk is low, when nutrients reserves and gut contents are low, and when host quality is low. Models also predict that host-feeding should decrease at older age. We use the terms "concurrent" when oviposition and host-feeding take place on a same host and "non-destructive host-feeding" when the host is still suitable for progeny development after the meal (Jervis et al. 1996). Most models apply to non-concurrent and destructive host-feeding but some include concurrent host-feeding. In one such model, tested with *Aphytis* parasitoids, the cost for the progeny developing in a host where host-feeding occurred was assumed to be 75% (Heimpel et al. 1994). Such costs occur because host-feeding decreases the resources available for the progeny whose size will likely be smaller resulting in a lower fitness (Ferracini et al. 2006). For a female, the indirect benefits of host-feeding must outweigh the immediate fitness cost (Burger et al. 2004).

Trichogramma wasps are moderately synovigenic species that emerge with some eggs but have the opportunity to produce more during their lifetime (Jervis et al. 2001). Female *Trichogramma* acquire proteins, amino acids and lipids from feeding on Lepidoptera eggs (Ferracini et al. 2006). *Trichogramma* spp. practice concurrent but non-destructive host-feeding The effect of host-feeding on longevity of *Trichogramma* spp. varies. Host-feeding increases longevity in presence of honey in *T. minutum* Riley (Hymenoptera: Trichogrammatidae) (Bai and Smith 1993), has a neutral effect in *T. embryophagum* Htg. (Klomp and Teerink 1967) and decreases longevity in absence of honey in *T. euproctidis* Girault probably because of the cost of producing more eggs (Ferracini et al. 2006). When female *Trichogramma* encounter a host, they can lay an egg and invest in current reproduction or lay an egg and host-feed thereby increasing their future reproduction but decreasing the value of the progeny they deposited in that host (Lebreton et al. 2009). In *T. euproctidis*, a significant reduction in progeny tibia length following host-feeding occur but that reduction is less than 5% (Ferracini et al. 2006).

Contrary to what is observed in most species (Heimpel et al. 1994; Collier 1995; McGregor 1997; Thompson 1999; Giron et al. 2002), *Trichogramma* females host-feed on the vitellus oozing from the oviposition puncture and therefore oviposit prior to host-feeding (Ferracini et al. 2006). In rare cases, host-feeding has been found to occur before ovipositing by inexperienced *Trichogramma* females on the first egg they encountered (Nurindah et al. 1999).

In this paper, we tested if age and hunger level of females influenced their host-feeding behaviour in *Trichogramma euproctidis*. Young and old females are expected to use different strategies for maximizing their reproductive success and should adjust their host-feeding behaviour in consequence. Young females, with a longer life expectancy, should host-feed more in order to invest in future reproduction. We also hypothesized that, if host-feeding is used for somatic maintenance, starved females should host-feed more than water-fed females, who should host-feed more than honey-fed females. The distribution of host-feeding bouts in a sequence of hosts encountered, the time taken to parasitize 10 different hosts, used as an indicator of female's mobility, and the sex ratio deposited were also investigated.

Material and methods:

Insect rearing

The *T. euproctidis* used in this study originated from Egypt (*T. evanescens* strain 20 in Godin and Boivin 2000). The culture was maintained at 25 ± 2 °C, 50% RH, and LD 16:8 h on eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). These conditions are near the optimum for this species (Carrière and Boivin 1997). The egg volume of this host usually allows for the development of a single *Trichogramma* (Bai et al. 1992). Females were isolated at emergence, mated on the day of emergence and were naïve (i.e. they did not have experience with host eggs other than the one from which they emerged). They were kept in Beem® polyethylene capsules in the same conditions as the rearing until tested.

Effect of age

In order to measure the effect of age on host-feeding behaviour, 19 newly emerged females received daily a drop of diluted-honey (50:50) and were kept for 5 days in their capsule before observation (old females). Another 18 females received a drop of diluted-honey (50:50) but were observed in the 10 hours following emergence (young females).

Effect of hunger

To assess the effect of hunger on host-feeding behaviour, 66 females were distributed among three treatments. In treatment 1, 22 females received a drop of diluted-honey (50:50), in treatment 2, 22 females were offered a drop of water and in treatment 3, 22 females had access to neither water or diluted-honey. The drops were placed the day before emergence to make sure females had the opportunity to feed as soon as they emerged. They were kept for 24 hours in their capsule before observations.

Observation

Trichogramma euproctidis females were individually offered a square patch of 16 fresh (less than 24 hours) *E. kuehniella* eggs on a filter paper of 12.5 mm diameter under laboratory conditions (25±2°C, 30±5% r.h.). The distance between each egg was 3 mm. The behaviours observed were oviposition, walking, resting and host-feeding. The behaviours were recorded using The Observer XT software, a camera and a binocular microscope. Observations started when the female parasitized its first host and ended after ten different hosts were parasitized. At the end of each observation on the effect of hunger, parasitized eggs were transferred into Beem® polyethylene capsules and incubated at 25 ±2°C, 50±5% r.h. and photoperiod of L16:D8 until emergence. Progeny wasps were then sexed. Only data from females that performed at least one host-feeding were kept for comparisons (zeros were excluded of the mean's calculation after verifying if the proportion of non-host-feeding females was not different between young and old females with a chi-square test). For that reason, three young females were discarded.

The following parameters were obtained: frequency (number of host-feeding bouts per observation), total duration (time spent doing host-feeding), number of host eggs used for feeding and time taken to parasitize 10 different host eggs (the duration of a sequence excluding host-feeding duration).

Statistical analysis

Statistical analyses have been done using R 2.11.1 software (R Foundation for Statistical Computing). Data did not follow normal distribution (Shapiro-Wilk normality test) and non-parametric statistics were used. Mann-Whitney tests have been used for comparisons between young and old females. Kruskall-Wallis tests were run to compare starved, water-fed and honey-fed females and if significant at $P<0.05$, a non-parametric multiple comparisons test between treatments was then used (package “pgirmess” and function “kruskalmc”). Chi-square tests were used to compare proportion of females and males emerging from eggs on which the mother host-fed and emerging from host eggs without host-feeding.

Results:

No significant difference in host-feeding frequency, duration, number of hosts used for host-feeding and time taken to parasitize 10 different hosts was observed between young and old females (Table 3.1). From the 18 young females observed, 15 were kept for the analysis since three young females did not host-feed. The proportion of young non host-feeding females was not significantly different from the number of old females that did not host-feed (0) (Chi-square test: 3 of 18, 0 of 19; $P= 0.210$).

Water- and honey-fed females host-fed for a shorter duration than starved females (Table 3.1). The time taken to parasitize a sequence of ten different hosts was longer for starved females than for water-fed females. However, no significant difference was observed in host-feeding frequency, number of hosts used for nutrition and in total F1 sex ratio.

The sex ratio (proportion of males) of the progeny that emerged from hosts with host-feeding was higher than in hosts without host-feeding for the complete sequence of 10 hosts encountered (Table 3.2). However, when we removed the first host from calculation, sex ratio was similar between hosts with and without host-feeding (Table

3.2). The majority of females (89%) host-fed on the first egg encountered where a male was generally allocated. Furthermore, 75% host-fed on more than one host and 85% had more than one occurrence of host-feeding in the sequence of 10 hosts.

Discussion:

Age did not affect the host-feeding behaviour contrarily to what was predicted. We found no difference between young and old females in the frequency, duration and number of host eggs used for nutrition. According to the predictions of a dynamic state-variable model (Heimpel and Collier 1996), host-feeding should decrease at older age, as females near the end of their life would gain little by host-feeding and investing in future reproduction (Heimpel and Rosenheim 1995). Furthermore, host deprivation (time spent without host) should decrease the parasitoid's perception of host availability (Rosenheim and Heimpel 1994) and females should host-feed less. In our experiment, older females have been 5 days in isolation without host and should have been less likely to use hosts for host-feeding. However, our results are in line with other studies that failed to verify this prediction. The prediction that older females are less likely to host-feed than younger females was not supported in *Aphytis melinus* DeBach (Hymenoptera: Aphelinidea) and *Aphytis aonidiae* Mercet (Heimpel and Rosenheim 1995; Heimpel et al. 1996) and in *Trichogramma australicum* Girault, (Nurindah et al. 1999). In the endoparasitoid *Itoplectis naranyae* Ashmead (Hymenoptera: Ichneumonidae), females have been found to increase their host-feeding behaviour but this behaviour was linked to an increase in oviposition in the days following emergence (Ueno and Ueno 2007). Female parasitoids can adjust their host preference, acceptance and patch exploitation strategy when they are aging (Weisser 1994; Goubault et al. 2005; Wajnberg et al. 2006; Lebreton et al. 2009), but still not be able to relate their life expectancy to their host-feeding behaviour. In addition, for *Trichogramma* spp., the cost of host-feeding may be low enough that aging females still gain more by host-feeding than by not doing so. Host-feeding does decrease progeny fitness in *Trichogramma* but when tibia length is used as proxy of fitness, the cost is less than 5%, in *Trichogramma turkestanica* (now *euproctidis*) (Ferracini et al. 2006). In addition, five days-old *Trichogramma* females are probably rare in the field and selection on the behaviour of these 'old' wasps may be weak or absent in nature (Heimpel and Collier 1996).

Mobility of parasitoid females can be used as an indicator of their fitness and their efficiency in host searching and parasitism (Bigler 1989; Dutton and Bigler 1995; Pompanon and Boulétreau 1997; Tezze and Botto 2004; Boivin 2010; Suverkropp et al. 2010). The mobility of old and young *T. euproctidis* females was similar although a decrease in activity and in linear walking speed with age have been observed in *T. brassicae* Bezdenko (Pompanon et al. 1999). However, *T. brassicae* females were observed at temperature of 22°C, until they reached seven days-old, which is two days older than our females. Their locomotor activities increased for 3-4 days and then started to decline (Pompanon et al. 1999). Our *T. euproctidis* females may see their mobility affected later in their live or when exposed to higher temperature.

A female's hunger level and nutrients reserve can strongly affect its behaviours and change its foraging decisions (Heimpel and Rosenheim 1995; Jacob and Evans 2001; Giron et al. 2002; Rivero and West 2005; Jervis et al. 2008; Lightle et al. 2010). As expected, starved females host-fed longer, but the frequency and number of host eggs used for nutrition were the same between the three groups. A higher frequency of host-feeding would result in more insertion of ovipositor and more investment in time and energy and a higher number of hosts used for host-feeding would have mean more progeny with a lower fitness, even if the cost is low. To host-feed longer but on the same number of hosts can thus be considered a good strategy for the females. However, we did not measure if starved females ingested more host material by increasing the duration of host-feeding. Starved females were weak and could have taken longer to ingest the same amount of host material.

We were expecting that water-fed females would host-feed more than honey-fed females as honey provides fuel for somatic maintenance and locomotion (Thompson 1999; Jervis et al. 2008) while it is poor in amino acids and lipids (Jervis et al. 2008). However, although water-fed females host-fed longer than honey-fed females, this difference was not significant. The fact that water-fed females host-fed less than starved females suggests that either host-feeding serves to acquire water or that the water ingested by the water-fed females took part of the volume of the gut and prevented the female from host-feeding as much as starved females. Host-feeding could be used by *Trichogramma* females to acquire water while exploiting a host patch. Producing eggs require water (Halima et al. 1983; Geister et al. 2008) and drinking or eating food containing water is necessary for females producing eggs to hydrate themselves (Danks 2000).

Honey is normally used for somatic maintenance and as an energy source for the muscular system (Pompanon et al. 1999; Jervis et al. 2008). We could therefore have expected honey-fed females to increase their activity level in the patch but our results showed that honey-fed females were not faster on a host patch. Only starved *T. euproctidis* females were less mobile compared with water-fed females (Table 3.1). The importance of water in locomotion has been demonstrated in other insects species such as *Locusta migratoria* Linnaeus (Orthoptera: Acrididae) (Raubenheimer and Gäde 1996) and *Nauphoeta cinerea* (Dictyoptera: Blaberidae) (Reynierse et al. 1972). Drinking water dilutes the heamolymph and contributes to the increase in activities and thus, in locomotion (Raubenheimer and Gäde 1996), the availability of water being somewhat more important than the availability of food (Reynierse et al. 1972).

Most females host-fed on the first host encountered and host-fed more than once in the sequence of 10 hosts (only three young females did not host-feed). These results suggest that *T. euproctidis* females have a pre-determined pattern of host-feeding and always host-feed on the first egg encountered in a patch and that, as they gradually exploit a host patch, they host-feed a second and sometimes a third time. Female *Trichogramma* adjusts their sex-ratio deposited according to the breeding structure of the parasitoid's population in a patch of host (Damiens and Boivin 2005). When exploiting alone a host patch, female *T. euproctidis* allocated a female-biased sex-ratio as predicted by the Local Mate Competition model (Hamilton 1967). However, the sex-ratio allocated to hosts on which the mother host-fed was superior to hosts where no host-feeding occurred (Table 3.2). It appears that this difference in the sex-ratio allocated was due to the sex-ratio deposited in the first host encountered as the sex-ratios allocated to host where host-feeding occurred or not was similar when the first host encountered was removed (Table 3.2). The sex ratio allocated was constant between treatments and therefore the hunger level of females did not change the sex ratio allocated in a sequence of 10 hosts encountered (Table 3.1).

In *Trichogramma*, egg laying occurs before host-feeding and therefore the sex-ratio decision is made before host-feeding. However, the sex-ratio allocated in the first host parasitized in a patch can also be influenced by the sex-ratio strategy used by the female. *Trichogramma* are known to use precise sex allocation (Luck et al. 2001) where a male is generally deposited first in a sequence of host encountered in order to decrease the probability of depositing a female-only progeny within a host patch (Godfray 1994). As the male-first strategy coincides with host-feeding on the first host, it is not possible to determine to which strategy the increase in the proportion of males deposited on the first host encountered responds. While depositing a male early in the oviposition sequence does decrease the risk of female-only clutches, to host-feed on a host in which a male was allocated is also less costly in fitness term (Charnov 1982; King 1987; Luck et al. 2001; Santolamazza-Carbone et al. 2007) and represents a strategic choice for the female. In addition, host-feeding on the first host encountered ensures the female to gain materials for egg production as soon as possible.

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Table 3.1: Effect of each treatment on host-feeding (HF) frequency, on HF duration, on the number of hosts used for HF, on time taken to parasitize 10 different hosts (Sequence duration) and on sex ratio in F1 generation. Mean (± SD) durations are expressed in second

	Frequency HF	Duration HF (sec)	Nb hosts used HF	Sequence duration (sec)	Sex ratio F1	N
Young ♀	7.40 ± 7.16	218.6 ± 179.0	2.87 ± 1.73	3231.2 ± 1229.7	NA	15
Old ♀	9.32 ± 7.67	263.7 ± 169.9	3.79 ± 1.90	3953.5 ± 1234.0	NA	19
Mann-Whitney test p-value	0.3365	0.4102	0.2645	0.0829		
Starved ♀	10.77 ± 8.32	745.4 ± 283.7^{a}	2.73 ± 1.58	2443.3 ± 560.2^{a}	0.27 ± 0.08	22
Water-fed ♀	7.85 ± 5.80	220.2 ± 128.9^{b}	2.68 ± 1.21	1777.1 ± 298.7^{b}	0.24 ± 0.09	22
Honey-fed ♀	8.59 ± 7.18	407.7 ± 256.9^{b}	3.00 ± 1.72	2052.7 ± 343.9^{ab}	0.25 ± 0.08	22
Kruskall-Wallis test p-value	0.399	1.18E-07***	0.827	7.20E-05***	0.546	

Table 3.2: Number of males and females emerging from eggs subjected or not to maternal host-feeding (HF). Chi-square tests have been used to compare the proportions of males and females from non-HF and HF host egg

Sequence of 10 hosts	Starved		Water-fed		Honey-fed		Total 3 treatments	
	HF	Ø HF	HF	Ø HF	HF	Ø HF	HF	Ø HF
Males	24	33	21	28	28	25	73	86
Females	31	121	35	122	38	121	104	364
Sex ratio	0.44	0.21	0.38	0.19	0.42	0.17	0.41	0.19
X2 test p-value	3.72E-03		8.27E-03		1.65E-04		1.79E-08	
Without first host	Starved		Water-fed		Honey-fed		Total 3 treatments	
	HF	Ø HF	HF	Ø HF	HF	Ø HF	HF	Ø HF
Males	8	33	6	28	12	25	26	86
Females	27	121	29	122	31	121	87	364
Sex ratio	0.23	0.21	0.17	0.19	0.28	0.17	0.23	0.19
X2 test p-value	0.966		0.974		0.178		0.426	

GENERAL CONCLUSION

The objectives of this study were: (1) to verify the effect of low temperature exposure during immature development on several fitness proxies and on host-feeding behaviour used as an indicator of energy level and (2) to examine the effect of age and hunger on the propensity of females to host-feed and on their mobility on host patch.

In the second chapter, results showed that low temperature exposure during immature development affected negatively fitness of adults. We obtained lower percentage of emergence, longevity and fecundity when *T. brassicae* were directly exposed to temperature of 5°C but acclimation decreased the fitness cost by allowing development to the prepupal stage, a developmental stage less sensitive to low temperature in *Trichogramma* (Garcia et al. 2002). However, exposing the maternal generation to low photoperiod did not reduce *T. brassicae*'s fitness cost as expected. These results confirm our first hypothesis stating that females *T. brassicae* fitness should be affected with temperature and duration of exposure but invalidate partly the second hypothesis stating that acclimation and maternal photoperiod should change the fitness cost associated with low temperature exposure. Only acclimation reduced the costs associated with low temperature exposure and 10°C during 30 days was the best acclimation treatment for *T. brassicae*. Since there was no difference in propensity of females to host-feed after low temperature exposure, we concluded that host-feeding cannot be used as an indicator of fitness loss (Hypothesis no3). The decrease in the energy reserve may not have been important enough to cause a difference in the female's host-feeding behaviour.

In the third chapter, we tested whether two female's states (age and hunger level) independently influenced the decision and propensity to host-feed in *Trichogramma* *euproctidis*. The distribution of host-feeding bouts in a sequence of host encountered, the female's mobility and the sex ratio deposited were also investigated. Age did not influence the host-feeding behaviour but hunger level did. Thirst may also be the reason why females *Trichogramma* performed host-feeding. Mobility was not affected by age or hunger level. However we found that host-feeding bouts happened more frequently on the first host egg where a male was generally allocated. Host feeding and depositing a male in the same host decrease the cost of host feeding as reducing the resource of an immature male results in a smaller adult male and smaller size is less costly in fitness term compared to a female (Charnov 1982, King, 1987, Luck et al. 2001, Santolamazza-Carbone et al. 2007). In addition, host-feeding on the first host encountered ensures resources for egg production as soon as possible. Dynamic state-variable models had made predictions for host-feeding behaviour, but they did not take into count the low cost (less than 5% reduction in tibia length (Ferracini et al. 2006)) associated with host-feeding in the case of a concurrent but non-destructive host-feeder such as *Trichogramma* spp. The only model for concurrent host-feeding used a higher cost (75%) with *Aphytis* species (Heimpel et al. 1994). The low fitness cost associated with host-feeding in *Trichogramma* may be the reason why females continued to host-feed when aging.

This study brings a better understanding of the behavioural ecology of parasitoids with low fitness cost associated with host-feeding and on *Trichogramma* species. This is the first study trying to use female's host-feeding behaviour as an indicator of its reserve of energy level after low temperature exposure. Our results indicate that the predictions of models for concurrent and non-destructive host-feeding should be modified when host-feeding cost is low as for *Trichogramma* species. Other females' physiological factors, such as egg load, mortality risk, egg maturation delay, inability to resorb eggs and egg resorption cost (Heimpel and Collier 1996), are likely to influence this behaviour but have not been tested in this study. This study brings additional information on the distribution of host-feeding behaviour in a sequence of hosts encountered. The first host parasitized is generally used for host-feeding and at least one another host is used for nutrition later in the sequence of hosts encountered. This strategy was previously described by Ferracini et al. (2006) but the possible link with sex allocation had never been discussed and tested before.

These results could be used to optimize mass rearing and biological control. Cold storage is commonly used by industries to synchronize production with the demands in the field and to conserve large stocks of insects (Jalali and Singh 1992, Smith 1996, Pitcher et al. 2002). Low temperature exposure treatment during the immature development of *Trichogramma* can therefore be improved by using an acclimation period at 10°C during 30 days for *T. brassicae*. Since there is an augmentation of the use of egg parasitoids (Parra 2010), it is important to understand the factors acting on the behavioural decisions linked with reproduction, and thus, their efficiency as biological control agents. The age, hunger and energy reserve after low temperature exposure are some of these factors and host-feeding behaviour represents an important decision between current and future reproduction for parasitoids. However, this study shows that these factors have limited impact on host-feeding since this behaviour had low costs in *Trichogramma*. While the factor of hunger was tested, the presence of water had more impact than expected first and should be considered in further studies to optimize rearing.

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