

Sex allocation and mating structure in the egg parasitoids of the genus  
*Trichogramma* (Hymenoptera: Trichogrammatidae)

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## ABSTRACT

Haplodiploid Hymenoptera females control the sex of their progeny, and their sex allocation is influenced by several factors. The impact of intra- and interspecific competition and of inbreeding and outbreeding on sex allocation has been studied in some species of the egg parasitoid *Trichogramma*. The pre-mating dispersion has also been studied.

Impact of competition on sex allocation was observed for *Trichogramma minutum* Riley and *Trichogramma pintoii* Voegelé. These species were chosen because of the ease with which they can be distinguished. Results show that females of both species lay more males under intraspecific competition than alone, following the Local Mate Competition theory, while only *T. pintoii* modifies its sex ratio under interspecific competition. Multiparasitism and natural habitat could explain this shift in the sex ratio.

*Trichogramma minutum*, *T. pintoii* and *Trichogramma evanescens* Westwood pre-mating dispersion show that most matings occur at the emergence site. However, the three species have a potential for off-patch mating, allowing genetic exchange between sub-populations. These three species were chosen because they are classified in different groups in the genus.

Finally, *T. evanescens* did not modify its sex ratio following inbreeding or outbreeding. The incapacity to discriminate between kin and non-kin, insufficient genetic distance in outbreeding, or the population structure could explain these results.

## RÉSUMÉ

Les femelles Hyménoptères haplodiploïdes possèdent la capacité de contrôler le sexe de leur progéniture selon différents facteurs. L'impact de la compétition intra- et interspécifique et des accouplements apparentés sur l'allocation des sexes, ainsi que la dispersion pré-copulatoire, ont donc été étudiés chez quelques espèces de parasitoïdes des œufs du genre *Trichogramma*.

Les résultats montrent que les femelles *T. minutum* et *T. pintoï* produisent plus de mâles en compétition intraspécifique que seules, alors que seule *T. pintoï* modifie sa proportion de mâles en compétition interspécifique. Le multiparasitisme ainsi que l'environnement naturel peuvent expliquer cette modification.

Ensuite, chez *T. minutum*, *T. pintoï* et *T. evanescens* la majorité des accouplements se font sur le site d'émergence, avant la dispersion. Cependant, ces trois espèces possèdent tout de même un potentiel d'accouplements à l'extérieur du site d'émergence.

Finalement, *T. evanescens* ne modifie pas sa proportion de mâles suite aux accouplements apparentés. L'incapacité de discriminer les parents, une distance génétique insuffisante entre non-apparentés et des populations structurées peuvent expliquer ces résultats.

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## CONTRIBUTIONS OF AUTHORS

All chapters are co-authored papers submitted or to be submitted. I have done all experiments, and written all papers. The co-author, Dr. Guy Boivin, has revised and corrected the papers before their submission. The second chapter, “Impact of intra- and interspecific competition on sex allocation by *Trichogramma* egg parasitoids”, has been submitted to *Entomologia Experimentalis et Applicata*. The third chapter, “Impact of inbreeding on sex allocation by *Trichogramma evanescens*”, will be submitted to *Environmental Entomology*, and the fourth chapter, “Impact of inbreeding and outbreeding in the egg parasitoid *Trichogramma*” has been submitted to *Biocontrol Science and Technology*.

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# **INTRODUCTION**

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Parent investment in their progeny is an important topic in behavioural ecology and several models have been developed to predict and explain the number of offspring and the sex ratio (proportion of males) produced by a female. Fisher's model (1930) predicts that when mating is at random in the population (panmixis), parents should invest the same amount of energy in each sex, leading to a 1:1 sex ratio. This equilibrium is present because there is no selective pressure pushing parents to produce more of one sex or the other. When a sex becomes rare, there is a frequency-dependent advantage to produce the rarer sex and, therefore, a genotype that produces an excess of this rarer sex will be favored by natural selection. As a result, equilibrium between the two sexes is obtained.

Hamilton (1967) later produced the Local Mate Competition (LMC) model that explains the occurrence of female-biased sex ratios in species with structured populations where mating occurs locally. In these populations, where mating occurs locally on a patch, sex ratios are female-biased to reduce competition for mating between kins. Females under LMC lay only as many sons as necessary to mate all their daughters. When there is more than one foundress on a patch (when multiple mothers are present), each female lays more sons than needed, increasing competition for mating between males. Such an increase in the proportion of males enhances the probability that the mother's genes will disperse. A number of authors have reviewed the LMC model (Nunney & Luck 1988; Hardy 1994; Flanagan et al. 1998) to include other factors like the dispersal of males and the synchronicity of foundresses on the patch (Nunney & Luck 1988). When the number of foundresses increases toward infinity, it leads the sex ratio toward the equality of Fisher.

In species with structured populations, inbreeding frequently occurs. After emergence, females have to choose between mating on the patch before dispersing or leaving the patch to find a mate. In gregarious or quasi-gregarious species, within-patch mating is frequent, while in solitary species, the females have to leave the host to find a mating partner because they are alone at emergence (Godfray 1994). Gregarious species can show protandry: males emerge first and wait for females, mainly their sisters, increasing chances of sibmating (Wiklund & Fagerström 1977). When sibmating occurs, daughters are more closely related to the mother than sons

(see inbreeding section of literature review, p.14). Because of this asymmetry, the daughters resulting from sibmating are more valuable for the mother's fitness than the daughters resulting from outbreeding. Therefore, it is predicted that females mating with sibs deposit a more female-biased sex ratio than outbred females (Greeff 1996). Inbreeding and number of foundresses are the main causes of the low proportion of males found in very structured populations.

The LMC theory considers mating structure as fully local; no mating occurs outside the natal patch. However, intermediate situations are probably often encountered in many parasitoid species. Partial local mating, "an intermediate mating structure, between panmixis and fully local mating", seems to be frequent (Hardy 1994). There is no direct evidence on the occurrence of partial local mating, but only indirect comparative evidences in species where both winged and wingless males occur (West & Herre 1998, Fellowes et al. 1999).

*Trichogramma* spp. are hymenopterous egg parasitoids from the Trichogrammatidae family. This group has been studied extensively mainly for three reasons: 1) the ease with which it can be mass-reared (Losey & Calvin 1995); 2) its large utilization in biological control (Li 1994), and 3) its genetic structure allowing many ecological models. Most *Trichogramma* species reproduce by arrhenotokous parthenogenesis; males are haploid and come from non-fertilized eggs and females are diploid and come from fertilized eggs. The fertilization of the egg is under behavioural control by the female, who can stop the egg at the spermatheca to permit fertilization by stored spermatozooids to lay a female (Gerber & Klostermeyer 1970; Cole 1981; Suzuki et al. 1984). However, there are also some parasitoid populations that are thelytokous; females only lay daughters without mating, by parthenogenesis (Stouthamer et al. 1990). When a female finds a suitable host, she can control two aspects: the number of eggs to lay and the sex ratio to produce. The results depend on many factors such as abiotic factors, host quality, maternal age, diet, size and competition (King 1987).

## **OBJECTIVES**

The purpose of this study is to examine the impact of intra- and interspecific competition and inbreeding on sex allocation, and to evaluate the mating structure in *Trichogramma minutum* Riley, *T. pintoi* Voegelé and *T. evanescens* Westwood. The obtained results will improve the understanding of offspring allocation in these parasitoids, and could be used to optimize their use in mass rearing and biological control. These three species were chosen because they belong to different groups inside the genus (Pinto 1998), so they cover a variety of *Trichogramma* variability. In addition, *T. evanescens* and *T. minutum* have a great potential in biological control (Smith et al. 1990; Li 1994).

## **HYPOTHESES**

Four hypotheses will be tested in this study:

1. *Trichogramma* should follow the LMC theory (lay more sons when females are in groups), but sex ratio (proportion of males) should not be influenced by interspecific competition.
2. A low proportion of unmated females should be dispersing to assure some genetic exchanges between sub-populations. However this proportion should be low because of the low probability of finding a mate outside the natal patch for the females.
3. Males should disperse later to mate with as many females as possible, and unmated females should disperse later than mated females to increase their probability of being mated locally, and disperse only if there is no possibility for them to mate.
4. Inbred females should lay a more female-biased sex ratio than outbred females, because of the asymmetry in relatedness between daughters and sons with their mother.

## **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 contribution of authors is mentioned at page v.



# **CHAPTER I**

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## **LITERATURE REVIEW**

## 1. OPTIMAL PROGENY AND SEX ALLOCATION

Animals generally produce Fisherian sex ratios (proportion of males) of 0.5: equal investment in each sex (Fisher 1930). When a sex becomes rarer, it also becomes more valuable and is then advantaged, so equilibrium is obtained (Fisher 1930). In addition, the mechanism of sex determination in diplodiploid animals is generally chromosomal (CSD), and imposes a constraint to sex allocation adjustment (Williams 1979; West et al. 2000). Vertebrates and other diplodiploids are therefore known for rarely exhibiting extreme skews in sex ratios (West et al. 2000). However, some studies on vertebrates have recently shown sex ratios adjustments. In birds, blue tit (*Parus caeruleus* [Passeriforma: Paridae]) females produce a higher sex ratio when their mates have important ultraviolet ornamentation, which is a viability indicator (Sheldon et al. 1999). Seychelles warbler (*Acrocephalus sechellensis* [Oustalet] [Passeriforma: Sylviidae]) females also modify their sex ratio by producing more daughters on a high quality territory to have helpers that increase their fitness on this type of territory (Komdeur et al. 1997), and this bias is caused by a supplementary production of females, not by a biased mortality (Komdeur 1996). In mammals, the red deer (*Cervus elaphus* [Artiodactyla: Cervidae]) sex ratio is linked to maternal dominance (Clutton-Broch et al. 1986). However, these examples are still exceptions and the mechanisms implied are not well known.

In haplodiploid insects, such as hymenopterous parasitoids, progeny and sex allocation are more likely to be variable. When a female parasitoid encounters a suitable host, she has to take two decisions in her reproductive strategy: how many eggs to lay and what sex ratio to produce (Waage & Ming 1984). These decisions are important because hosts contain a limited amount of resources for the developing progeny and this amount will determine their survival and fitness once adults (Waage 1986). Progeny allocation depends on several factors: costs in time for laying an egg, rate of host finding, mortality, egg limitation, fitness realized by egg, female foraging experience, female age, egg load, and host quality and density (review by Waage 1986). The number of larvae developing per host influences adult size (Rabinovich 1971; Waage & Ming 1984) and adult size influences fitness through fecundity and longevity (Waage & Ming 1984). Therefore, females of gregarious species generally

lay more eggs in larger hosts, and when hosts are rare (Hardy et al. 1992; Vet et al. 1994; Mayhew & Godfray 1997).

In haplodiploid parasitoids, sex ratios vary from slightly male-biased to all-females. These variations are under female control when the reproduction involved is arrhenotokous haplodiploid parthenogenesis. Males are haploid and come from unfertilized eggs while females are diploid and come from fertilized eggs. Egg fertilization is under behavioural control by females; some eggs stop at the level of the spermatheca and are fertilized while those that do not stop are not fertilized. This behaviour enables an observer to sex the egg at oviposition; continuous abdominal movement results in a male and interrupted abdominal movement results in a female (Gerber & Klostermeyer 1970; Cole 1981; Suzuki et al. 1984). Interruption in movement corresponds to the moment when the egg is at the level of the spermatheca. Sex allocation, like progeny allocation, depends on many factors: parental characteristics, environmental characteristics, host characteristics and competition.

### **Parental characteristics**

The female mating status has a major influence on the sex ratio she will produce. As unfertilized eggs develop in males, virgin females will produce all male clutches. Delay before mating is also an important factor to sex allocation. If males' frequency is low in the population, the delay before being approached by a male will certainly be higher for females, and more females will stay unmated. Later mated females can then adjust their sex ratio in two ways. First, they can perceive that the population sex ratio is low, so that males are more valuable, and they will then lay more males (Werren & Charnov 1978). In opposition, some females will lay more females to compensate the effect of the large number of virgins on the sex ratio of the next generation (Hoelscher & Vinson 1971; Rotary & Gerling 1973). However, in both cases, if the delay before fecundation is long, oviposition of unfertilized eggs will be higher and it will increase the lifetime sex ratio of such females. Another important factor in females' status is the number of matings. Monoandrous species, where females mate only once, could become sperm depleted if the number of eggs laid is higher than the number of spermatozoa transferred by males. The sex ratio will

then increase because of the unfertilized eggs laid after sperm depletion. Mating with an empty male will also cause the female to stay technically virgin all her life and produce only males. However, in polyandrous species, females could also have a high sex ratio if the numerous spermatophores block the way of the spermatheca to spermatozoa (Flanders 1946).

Parasitoid age can modify sex allocation; older females generally produce more males because sperm stock and viability decrease. This is the case in *Telenomus heliothidis* Ashmead (Hymenoptera: Scelionidae) (Strand 1988) and *Lysiphlebus delhiensis* (Subba Rao & Sharma) (Hymenoptera: Aphidiidae) (Srivastava & Singh 1995). In addition, male age also influences sex ratios produced by the female, because older males transfer less sperm at mating and sperm viability decreases with age. Older males then induce the deposition of higher sex ratios in mated females (Srivastava & Singh 1995).

Oviposition sequence is also an important factor in sex allocation. Many species have precise sex allocation; males are laid early in the oviposition sequence, generally at the first or second oviposition. This is the case in *Trichogramma brassicae* Bezdenko (Wajnberg 1993), *T. chilonis* Ishii (Suzuki et al. 1984), *T. evanescens* (Flanders 1935), *T. pretiosum* Riley (Boivin, unpublished data), and *Telenomus heliothidis* (Strand 1988). Precise sex allocation changes sex ratios depending on the total number of eggs laid; as males are laid first, the smaller the clutch, the fewer females are produced (Suzuki et al. 1984).

Female size can also modify the sex ratio they deposit; larger females generally produce a lower sex ratio (Sagarra et al. 2001). Finally, maternal diet can influence sex ratios (Leatemia et al. 1995), but this factor could also be linked to age and longevity, as fed females live longer.

## **Environmental characteristics**

Some environmental factors change sex allocation by parasitoid females. The effects of temperature, humidity and photoperiod on sex ratios changes from one species to another. Temperature has various effects on sex ratios; it can increase (Smith & Hubbes 1986; Lysyk 1998, 2000), decrease (Hoelscher & Vinson 1971; van

Huis et al. 1994; Deng and Tsai 1998) or have no effect on it (Al-Maliky & Al-Izzi 1990; Chabi-Olaye et al. 2001). Relative humidity seems to have a negative relationship with sex ratios (Naganagoud & Kulkarni 1999), or to have no impact on it (van Huis et al. 1994). However, humidity is generally linked to temperature. Finally, there is limited evidence for a positive effect of photoperiod changes on sex ratios. Extreme photoperiod (between 0L:24D-10L:14D, and 14L:10D-24L:0D [hours of light : hours of darkness]) increases sex ratios in *Campoletis perdistinctus* (Viereck) (Hymenoptera: Ichneumonidae) (Hoelscher & Vinson 1971). In *Pteromalus puparum* (Linnaeus) (Hymenoptera: Pteromalidae), females under 10L:14D produced two to three times more females than under 14L:10D (Bouletreau 1976). These effects of abiotic factors could be a response to seasonal signals.

## **Host characteristics**

The host itself is an important factor influencing sex ratios. Its size, age, sex, species and density could have an impact on sex ratios. All these aspects can be grouped under "host quality".

### **a) Host Quality model**

#### ***Host size***

Hymenopteran sex ratios have been observed to change with host size in many species (Chewyreu 1913; Holdaway & Smith 1933; Brunson 1937; Clausen 1939; van den Assem 1971). A model has been developed to predict how sex ratios should vary with host size: the Host Quality model (Charnov 1979; Charnov et al. 1981). In fact, females evaluate the quality of the host and allocate the sex that will gain more fitness in it. As a larger host allows the development of a larger wasp, the obtained sex ratio will depend on the relation between size and fitness in each sex. Obviously, both sexes gain by being larger (Charnov et al. 1981). In females, size influences fitness by affecting longevity, egg supplies or searching efficiency (Godfray 1994). In males, size influences fitness by affecting longevity, ability to locate females and success in male-male competition (Godfray 1994). However, females gain more

fitness by increasing their size than males do (van den Assem 1971, Werren & Simbolotti 1989; Ueno 1999). Indeed, females are preferentially laid in larger hosts and males in smaller hosts (van den Assem 1971). However, it is not the absolute size of the hosts that is important, but rather its relative size in the environment, and its abundance proportion (Charnov 1979). This model has been verified for many species: *Lariophagus distinguendus* Först (Hymenoptera: Pteromalidae) (Werren & Simbolotti 1989), *Lariophagus* (Pteromalidae) (Charnov et al. 1981), *Heterospilus prosopidis* Viereck (Hymenoptera: Braconidae) (Ikawa et al. 1993), *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae) (King & King 1994), *Metaphycus stanleyi* Compere (Hymenoptera: Encyrtidae) (Bernal et al. 1998), *Pimpla nipponica* Uchida (Hymenoptera: Ichneumonidae) (Ueno 1999).

### ***Host age, stage, species and sex***

The Host Quality model of Charnov applies to factors influencing quality such as host age, stage, species and sex (Charnov et al. 1981). In many Trichogrammatidae species (around 21 species), females prefer younger hosts and, when gregarious, lay a smaller clutch size in older ones (Godin & Boivin 2000). For these egg parasitoids, younger hosts represent more resources than older ones. However, for larval parasitoids, older hosts are generally larger, so they represent more resources than younger larvae and more daughters are laid inside these hosts (Avila & Albajes 1984). In fact, host size is generally correlated with host age and developmental stage. As different species may have different sizes, host species will also have an impact on the sex ratio produced. Finally, host sex could influence sex ratios because it is linked to host size; females are generally larger than males in insects and their reproductive system represents more resources for developing parasitoids (Kraaijeveld et al. 1999).

### ***Superparasitism***

Superparasitism occurs when a female parasitoid oviposits in an already parasitized host. It has long been considered as a consequence of failure to discriminate (Fiske 1910; van Lenteren 1981; Tillman & Powell 1992), because

parasitized hosts represent a lower quality host (Skinner 1985). However, superparasitism could be advantageous under some circumstances. For example, if it reduces the effectiveness of the host immune system (Askew 1968a; Puttler 1974; Bakker et al. 1985). In most species, female parasitoids generally discriminate between parasitized and unparasitized hosts (van Lenteren 1981; van Alphen & Nell 1982; van Alphen et al. 1987; van Dijken & Waage 1987; King & Skinner 1991; Tillman & Powell 1992). A distinction is made between self-superparasitism, where a female parasitizes a host she has already parasitized, and conspecific superparasitism, where a female oviposits in a host parasitized by a conspecific female (Waage 1986). Self-superparasitism is expected to be less common in solitary species, except if it increases the probability of survival of one immature, by decreasing effectiveness of the host immune defense for example (Puttler 1974; Bakker et al. 1985). For conspecific superparasitism, it should be more frequent when the proportion of unparasitized hosts is low, but decreases as hosts become less suitable because of the presence of too many immatures or because of their age (Waage 1986). Because of their low quality, parasitized hosts receive smaller egg clutches (Wylie 1965; Holmes 1972; Werren 1980; Ikawa & Suzuki 1982; van Dijken & Waage 1987). Host Quality model also predicts a higher sex ratio in these low quality parasitized hosts (Charnov et al. 1981). Although it is the case in some species (Suzuki & Iwasa 1980; Werren 1980), the prediction fails for others (van Dijken & Waage 1987).

## **Competition**

When populations are structured, mating is no more at random, and the sex ratio is no longer the Fisherian 0.5 (Fisher 1930), because the panmixis assumption is broken; offspring mate among themselves on their natal patch. When a female is alone on a patch, she should produce only enough sons to mate all her daughters (Hamilton 1967). This sex ratio is adaptive because superfluous males will increase competition among brothers and will take the resources of potential females. In fact, female-biased sex ratios have evolved to decrease competition among sons (Courteau & Lessard 2000). So females of gregarious species will lay only one male per clutch

as long as the male could fertilize all his sisters (Griffiths & Godfray 1988). If the clutch is too large for the insemination capacity of one male, or if the risk of male immature mortality is high, more than one male will be laid (Heimpel 1994; Nagelkerke & Hardy 1994). Finally, if hosts are aggregated, it is more optimal for the female not to lay a male in each host because mating will occur between offspring of all aggregated hosts (Waage & Ming 1984).

Indeed, structured populations are common in hymenopteran parasitoids, and the sex ratio produced by females from these populations will change according to the number of foundresses present on a patch. This is called the Local Mate Competition (LMC) model (Hamilton 1967). Following this model, if only one female is present, she will lay the minimum number of sons required. However, if more than one foundress is present on the patch, offspring of each female will compete for mates. Each female will lay more males to increase the probability that her daughters will be mated by her sons, and that her sons will have some chance of mating other females. This behaviour increases mother fitness. Many parasitoid species follow the LMC model (see Herre et al. 1997 for a review), like *Trichogramma evanescens* (Waage & Lane 1994), *Telenomus remus* Nixon (Hymenoptera: Scelionidae) (van Welzen & Waage 1987), *Lariophagus distinguendus* (Werren & Simbolotti 1989), *Leptopilina heterotoma* (Thomson) (Hymenoptera: Eucollidae) (Debout et al. 2002) and *Spalangia cameroni* (King 1996).

To follow the LMC model, a female must perceive that she is not alone on a patch. Several mechanisms have been proposed: physical interference with other females (Wylie 1976, 1979; van Welzen & Waage 1987), perception of chemical traces (van Welzen & Waage 1987), and the rate of encounters of parasitized hosts versus unparasitized hosts (van Welzen & Waage 1987). In addition, two mechanisms are proposed for the modification in sex allocation: interference (Waage 1982) and change of sequence in oviposition (van Welzen & Waage 1987). In the interference mechanism, the average clutch size is smaller when females are in groups, and in species with precise sex allocation where males are laid first, this results in a higher sex ratio. The second mechanism can also modify the obtained sex ratio by allowing more males first.



The Host Quality model and the Local Mate Competition model have been combined together in a unique model (Werren 1984). This was necessary because the effects of both models cannot always be separated. When there is more than one foundress on a patch, the LMC model is appropriate. However, under these conditions, the proportion of already parasitized hosts is higher than when a female is alone, so the Host Quality model is also appropriate. Obviously, as the two models predict the sex ratio to shift toward more males, the predicted trend is the same.

**a) Interspecific Competition**

The impact of competition with females from another species on sex allocation has been less documented but the LMC model does not predict an increase in the number of sons, as the sons of a different species should not compete with the mates of a given species. Opposite results have been found for different species. As predicted, *Spalangia cameroni* does not increase its sex ratio when in presence of *Muscidifurax raptor* Girault & Saunders (Hymenoptera: Pteromalidae) (King 1996). However, *Muscidifurax raptor* females increase their sex ratio when on a patch with *Spalangia cameroni* (King & Seidl 1993), and *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) does so with *Spalangia cameroni*, but not significantly (Wylie 1976). In fact, most studies on interspecific competition observed the resulting densities of each population instead of sex allocation (Sasaba 1966; Strand et al. 1990; Ryoo et al. 1996; Lale & Vidal 2001).

**b) Partial local mating**

In Fisher's theory (1930), panmixis is the mating structure, and in Hamilton's LMC theory (1967), mating occurs fully locally. However, partial local mating, an intermediate mating structure where a proportion of mating occurs locally and some mating occur outside the patch is probably common (Hardy 1994). This should decrease the degree of competition among males, so species with such a mating structure are expected to produce a less female-biased sex ratio (Frank 1986; Nunney & Luck 1988; Taylor 1993). Partial local mating has been mainly studied through

species with dimorphic males: winged and wingless. In non-pollinating fig wasps, some species have wingless males so mating is fully local, and some species have winged males, so mating can occur both before, or after dispersion (West & Herre 1998). Species with wingless males (where mating is local) have a lower sex ratio than species with winged males (where out-patch mating is possible) (West & Herre 1998; Fellowes et al. 1999). When both forms are present (winged and wingless males), the sex ratio of these populations is intermediate (Fellowes et al. 1999). These results suggest that intermediate situations lead to intermediary sex ratios.

## **2. MATING STRUCTURE**

### **Inbreeding**

In animals, when an individual is sexually mature, he can mate with any opposite sex member of the population to procreate. However, in diploid species, sibmating increases risks of having homozygous deleterious recessive alleles that decrease fitness (King & King 1995). This is called inbreeding depression. Therefore, inbreeding behaviours are heavily penalized and natural selection instead favors inbreeding avoidance behaviours in such species (Ralls et al. 1986; Pusey 1987; Blouin & Blouin 1988; King & King 1995). Several processes can increase inbreeding avoidance, dispersal being one of these (Pusey 1980; Pusey & Packer 1987). In most primates, a sex-biased natal dispersal occurs before breeding (Pusey & Packer 1987). However, it is not the only reason why all birds and mammals disperse (Ralls et al. 1986). Another way to avoid inbreeding is to recognize kin and avoid mating with them (Wu et al. 1980; Bateson 1982; Kareem & Barnard 1982; Hepper 1983).

Even if inbreeding is avoided in many species, particularly in diploid species, outbreeding also incurs a cost: outbreeding depression. When outbreeding need dispersal, it means costs in energy and time, and it increases risk of predation during travel. Outbreeding depression can also occur if individuals from geographically separated population mate together. In this case, each individual can

be very adapted to its habitat, which can be different from the habitat of its partner, and the produced progeny can be misadapted to both habitats (Templeton 1986).

Nevertheless, in haplodiploid species where males are haploid and females are diploid, inbreeding depression is generally absent or at low level (Brückner 1978; Werren 1993; Antolin 1999). As males are haploid, deleterious recessive alleles are directly exposed to natural selection (King et al. 1969; King & King 1995). The only risk is about female-limited traits, like fecundity (King & King 1995). For parasitoid wasps in nature, inbreeding occurs in gregarious and quasi-gregarious species where relatives emerge together in the same host or patch of hosts (van den Assem et al. 1980). Mating generally occurs at least partially locally, therefore between offspring of all foundresses. The lower the number of foundresses, the higher the level of inbreeding (Frank 1985). In fact, inbreeding is generally considered as the rule in haplodiploid species (Hamilton 1967; Askew 1968b).

In most species, both inbreeding and outbreeding occur, with the proportion of inbreeding being generally higher than outbreeding. No inbreeding depression has been found in *Spalangia cameroni* (King & King 1995), *Cothonaspis bouleardi* (Hymenoptera: Eucoilidae) (Biémont & Bouletreau 1980), and *Dinarmus vagabundus* (Timberlake) (Hymenoptera: Pteromalidae) (Rojas-Rousse et al. 1988). Inbreeding generally occurs in *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) (Wilson 1961), a protandrous species where 81.3% of females mated locally shortly after emergence and are not very active for dispersion after emergence and mating (Loch & Walter 2002). The other 18.7% of females will be able to mate with offpatch mates because males are more active, are able to disperse and are sexually active all their life (Loch & Walter 2002). Males will then be able to find virgin females on their emergence sites. In three populations of *Trichogramma sp. A* and *T. pretiosum*, 55-64% of sibmating occurred and off-patch mating happened 31-33% of the time for *T. sp. A*, and only 2% in *T. pretiosum* (Kazmer & Luck 1991). In fact, outbreeding can occur in any species where males are able to disperse, where males or females are able to attract or locate individuals from the other sex, or are able to locate parasitized hosts to mate with the emerging females (Loch & Walter 1999). In *Spalangia cameroni* (Myint & Walter 1990), and *Pachycrepoideus vindemiae* Rondani

(Hymenoptera: Pteromalidae) (Nadel & Luck 1992), males are able to locate parasitized hosts.

Only a few species show inbreeding avoidance behaviours. One of these is *Bracon hebetor* Say (Hymenoptera: Braconidae), where diploid sterile males occur because of inbreeding (Whiting 1943). Individuals avoid sibmating by flying away (Whiting 1960; Antolin & Strand 1992; Ode et al. 1995), and avoiding mating immediately after emergence (Ode et al. 1995). In addition, females are capable of kin discrimination (Ode et al. 1995). Negative assorting mating, and asynchronous emergence are other ways to avoid inbreeding in species with inbreeding depression (Grant et al. 1980; King & King 1995; Debout et al. 2002).

The genetic structure of haplodiploidy induces an asymmetry in the relatedness of a mother with its progeny. Each individual receives a copy of its mother's genes, but only daughters receive a copy from their father. So both daughters and sons share 50% of the mother's genome, while daughters have 100% of the genes of their haploid father. Therefore, if a female and a male share common genes (as in inbreeding), the relatedness of the daughters to their mother will be more than 50% while the relatedness of sons with their mother will remain at 50%. Because of this asymmetry, daughters become more valuable to their mother under sibmating. Sex ratios produced from sibmating is then expected to be more female-biased (Herre 1985). In haplodiploid pollinator fig wasps, species with a high level of inbreeding have a lower sex ratio (Herre et al. 1997). However, in *Leptopilina heterotoma* (Hey & Gargiulo 1985), and *Spalangia cameroni* (King and King 1995), no effect of inbreeding on sex ratios has been observed.

#### **a) Mate discrimination**

Parasitoid males generally possess no mate discrimination ability (Matthews 1975), so that role is attributed to the females. It seems that females are able to discriminate many differences among males, and that olfactory cues are important in this recognition (White & Grant 1977; Ode et al. 1995).

One of the males' aspects which is important for females to discriminate is the degree of relatedness. Depending on the presence and degree of inbreeding

depression, females must evaluate this relatedness. This ability is found in several insect species. The female of the field cricket *Gryllus bimaculatus* (de Geer) (Orthoptera: Gryllidae) is able to discriminate between sibling and non-sibling mates; probability of successful mating and time passed with a partner is higher with non-related mates or cousins than with brothers (Simmons 1989, 1991). A pheromone is implicated in this discrimination (Simmons 1989), and the distance from emergence site when mating is also important, giving cues on the relatedness of males (Simmons 1991). In *Drosophila* (Diptera: Drosophilidae), olfactory cues are also used by females to discriminate between kin and non-kin mates (Ehrman 1969). A frequency-dependent mating is adopted: the rarer genotypes have more chances of successful mating (Ehrman & Probbler 1978). This strategy enables females to increase their chances of outbreeding. In fact, female *Drosophila* generally rejects the first male to court them and will accept males with phenotypes different from this first male (Spiess 1987). This mate discrimination ability is also found in some parasitoids. In *Bracon hebetor*, a species with inbreeding depression, the female has the ability to recognize and discriminate males from the same brood and avoid mating with them for five days (Ode et al. 1995). However, related males from other broods are not recognized, and therefore accepted.

## **Virginity**

In diploid species, virginity reduces the offspring production in the population, but has no impact on the population sex ratio. In arrhenotokous species, virginity implies constrained sex ratios; as unfertilized eggs produce males, unmated females will produce all-males broods, but with the same clutch size (Ode et al. 1997). Presence of ovipositing females in the population will then lead to less female-biased sex ratios. This change in the population sex ratio creates a selective pressure on mated females to produce a more female-biased sex ratio (Godfray 1990; Hardy & Godfray 1990; Heimpel 1994). Effectively, when the delay between emergence and being approached by a male is high, females mated by these males will produce a lower sex ratio (Hoelscher & Vinson 1971; Rotary & Gerling 1973). In opposition, in species with no virgin oviposition, such females will produce more males because as

males are rarer, they are also more valuable (Werren & Charnov 1978). Although these females can adjust their sex ratio toward females because of the higher frequency of virgin females, mated females are not able to evaluate the proportion of virgin females in the population (Ode et al. 1997). In addition, if mated females adjusted their sex ratio following the proportion of virgin females in the population, there is no pressure on virgin females to find a mate to adjust their own sex ratio (Godfray 1990).

In structured population, where local mate competition is high, virginity is more disadvantageous than in panmictic population because females cannot produce the optimal female-biased sex ratio and have a lower fitness (Godfray 1990; Godfray & Hardy 1993). This disadvantage would tend to lead to a lower level of virginity for such species, and penalize virginity heavily (Godfray 1990; Hardy & Godfray 1990; Godfray & Hardy 1993). However, there is more virginity in gregarious species where the LMC is higher than in solitary species (Godfray & Hardy 1993). This high level of virginity can be caused by: 1) differential mortality (Green et al 1982; Nagelkerke & Hardy 1994); 2) limited male insemination capacity or temporary sperm depletion (Sekhar 1957; Schlinger & Hall 1960; Wilson 1961; Gordh & DeBach 1976; Nadel & Luck 1985; Godfray 1990); 3) emptiness of the female spermatheca (Godfray 1994). In fact, the level of virginity changes between generations because of the dependence of males' efficiency to abiotic and biological factors (Godfray & Hardy 1993). To decrease the level of virginity, different strategies have evolved. Females can lay a higher sex ratio (Nagelkerke & Hardy 1994); some females are laying an additional "insurance" son to increase the sex ratio and avoid mortality that can eliminate every son (Debout et al 2002). However, this second son is wasteful if the mortality rate is low, and its cost is higher if clutch size is small (Heimpel 1994), but the disadvantage of superfluous males is lower than the disadvantage of virginity in large clutches (Hardy & Cook 1995). Precise sex allocation can also decrease the level of virginity, by assuring that there will be at least one male in each clutch (Green et al. 1982).

Constrained oviposition has several effects on populations: 1) it influences evolution of mating systems (Godfray & Hardy 1993); 2) it reduces spread of

parasitic chromosomes (Werren & Beukeboom 1993); 3) it facilitates evolution of eusociality (Godfray & Grafen 1988). So presence of virgin females has a great impact on parasitoid populations.

## **Dispersion**

Dispersal is here considered as any one-way movement of individuals from their emergence site (Lidicker & Stenseth 1992). Many intrinsic and extrinsic factors can lead to dispersal. Intrinsic factors include reproduction (inbreeding avoidance or mate competition avoidance between kin), and lack of territories in territorial species (Lidicker & Stenseth 1992). In extrinsic factors are found the need for a more suitable habitat (because of fire, erosion, floods, lack of resources or lower ability of some individuals to extract them from the habitat), social mechanisms, and interspecific interactions (competition, predation, and parasitism) (Lidicker & Stenseth 1992).

In insects, dispersal also depends on abiotic factors: temperature, wind (McManus 1988; Fournier & Boivin 2000), photoperiod (McManus 1988; Roderick & Caldwell 1992), and solar radiation (Fournier & Boivin 2000). Dispersal also depends on the biology of the parasitoid. A gregarious parasitoid attacking an abundant and aggregated host should have less capacity to disperse than a solitary species attacking solitary hosts (Fournier & Boivin 2000).

In *Trichogramma*, dispersion is expected to occur soon after emergence because of proovogeny, protandry, rapid and local mating, and arrhenotoky (Pompanon et al 1995).

### **a) Pre- or post- mating dispersion**

In many haplodiploid species, mating is considered as occurring locally, before dispersion. Adult emergence is generally synchronous (Pompanon et al 1995) and many species show protandry (Pompanon et al 1995; Doyon & Boivin, unpublished data.). Protandry, where males emerge before females, increases the probability of local mating (Wiklund & Fagerström 1977). In addition, delay between emergence and the first locomotory activity is different between males and females;

males start moving earlier than females (Forsse et al. 1992; Pompanon et al. 1995). Therefore, most males are active during females' emergence, and the low mobility of females favor encountering and mating on site (Pompanon et al. 1995). Males also fly less than females in *Trichogramma*, and this is even truer if there are still females on the patch (Forsse et al. 1992). In some species, males can discriminate between mated and unmated females (van den Assem 1986), and are attracted by virgin females (Pintureau & Toonders 1983; Fauvergue et al. 1995). Males remain on a patch as long as there are females, particularly virgin ones. As mating enhances the propensity to move in females (Forsse et al. 1992; Pompanon et al. 1995), mated females are more subject to dispersion than virgin ones. Because of this, there are only few virgin females dispersing in the wild, at least in *Trichogramma* spp. (Pompanon et al 1995). In fact, there is between 2-33% of off-patch mating in *Trichogramma* in field (Kazmer & Luck 1991).

### **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 & Gaston 1990). Therefore, opposite to a parasite, the parasitoid uses only one host and necessarily kills it. Most parasitoids are arthropods.

#### **Description**

Parasitoids can attack all host stages: eggs, larvae, pupae and adults. They can be classified as endo- or ectoparasitoids, depending if they develop inside or outside their hosts. Some parasitoids are idiobionts, and kill or permanently paralyze their hosts before their eggs hatch, and others are koinobionts, and kill their hosts only after their development (Haeselbarth 1979; Askew & 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. When hosts are aggregated, but parasitoids are solitary, these parasitoids are called quasi-gregarious



(van den Assem et al. 1980). In insects, there are around 68 000 known parasitoid species on about 850 000 insect species distributed in several orders: Lepidoptera (11 species), Trichoptera (1 species), Diptera (15 000 species), Coleoptera (3 000 species), Neuroptera (50 species), and Hymenoptera (50 000 species) (Godfray 1994).

## **Trichogrammatidae**

The Trichogrammatidae is a hymenopterous Chalcidoidea family. Approximately 260 species, distributed in 80 genera are known (Pinto & Stouthamer 1994). They are all arrhenotokous or thelytokous parasitoids. They are found worldwide in the six biogeographic regions (Pinto & Stouthamer 1994). Species of Trichogrammatidae are solitary or gregarious endoparasitoids of insect eggs (with a few exceptions like puparia of Cecidomyiidae (Viggiani 1981)) (Pinto & Stouthamer 1994).

### **a) Trichogramma**

*Trichogramma* is the largest genus of the Trichogrammatidae with around 145 known species (Pinto & Stouthamer 1994). The fact that more species are described in this genus is probably linked to their importance in biological control. All terrestrial habitats that have been sampled contained *Trichogramma* species. Their distribution follows this pattern: Palearctic (50 species), Oriental (35 species), Nearctic (28 species), Neotropical (24 species), Afrotropical (8 species) and Australian (7 species) (Pinto & Stouthamer 1994). The genus is divided into two subgenera: *Trichogramma* and *Trichogrammanza*; the latter one contains only three Australian species. The species used in the experiment for this thesis are *Trichogramma evanescens*, *T. minutum* and *T. pintoi*. As mentioned in the introduction, this choice have been made because they all three belong to different groups inside the genus (Pinto 1998), so they cover a variety of *Trichogramma* variability. In addition, *T. evanescens* and *T. minutum* have great potential for biological control (Smith et al. 1990; Li 1994). *Trichogramma pintoi*, which is less studied and used for biological control, have been chosen for the experiment on

interspecific competition because it can be easily distinguish from *T. minutum* by the color of its body.

### ***Trichogramma evanescens* Westwood**

*Trichogramma evanescens*, a brown species (Fig 1.1A) is found in Europe, Asia and Africa (Thompson 1958). This egg parasitoid is found in field habitats where it attacks hosts on grasses and herbaceous plants (Flanders 1937). Some of the lepidopterous host's genera attacked by *T. evanescens* are *Hyphantria* (Arctiidae), *Sitotroga* (Gelechiidae), *Bupalus* (Geometridae), *Malacosoma* (Lasiocampidae), *Lymantria* (Lymantriidae) (Thompson 1958; Anderson 1976).

On *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), the rearing host, at 25°C and 16L:8D, females live 84.7 hours and produce 55.6 eggs with a sex ratio of 0.2 (Boivin & Lagacé 1999). Their developmental time from egg to adult is 13.54 days at 25°C (Bourarach & Hawlitzky 1989).

*Trichogramma evanescens* is commercially used against stored product moths in Europe (Schöller & Prozell 2002).

### ***Trichogramma minutum* Riley**

*Trichogramma minutum* is a yellow species (Fig 1.1B) found in eastern North America and Mexico (Pinto 1998). These parasitoids live in both natural and disturbed habitats, and attack hosts living on trees or herbaceous plants (Pinto 1998). They are polyphagous and attack a large range of hosts, mainly Lepidoptera, such as *Diatraea* (Crambidae), *Malacosoma* (Lasiocampidae), *Heliothis* (Noctuidae), *Heterocampa* (Notodontidae), *Basilarchia* (Nymphalidae), *Danaus* (Nymphalidae), *Manduca* (Sphingidae), *Choristoneura* (Tortricidae), *Cydia* (Tortricidae) (Anderson 1976; Pinto 1998).

On *E. kuehniella*, the rearing host, at 25±0.5°C, 50±5% RH and 16L:8D photoperiod, unfed females live 3.7±1.4 days and produce 83.6±24.5 progeny with a sex ratio of 0.246 (Leatemala et al. 1995). The majority of the oviposition occurs in the

first days. The developmental time from egg to adult is around 8 days (Yu et al 1984; Smith & Hubbes 1986).

*T. minutum* is used in biological control of the spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) in North America (Houseweart 1985; Smith et al. 1990).

### ***Trichogramma pintoi* Voegele**

*Trichogramma pintoi* is a black holarctic species (Fig. 1.1C) found in North America, Palearctic and West Indies (Pinto 1998). In North America, *T. pintoi* has been found on the following Lepidoptera: *Icaricia acmon* Westwood & Hewitson (Lycaenidae), *Lycaeides melissa* Edwards (Lycaenidae), *Actebia fennica* (Tauscher) (Noctuidae), *Vanessa* (Nymphalidae), and *Platyptilia carduidactyla* Riley (Pterophoridae) (Pinto 1998).

On *E. kuehniella*, the rearing host, at 25±1°C, 70±5% RH and 14L:10D, fed males live 2.5 days and fed females live 9.5 days and lay 38.6 offspring with a sex ratio of 0.408. The developmental time from egg to adult is 11.3 days (Cabello & Vargas 1987). On the cotton bollworm *Heliothis armigera* (Hübner) (Lepidoptera: Noctuidae) at 26°C and 60% RH, unfed females live 4.5-5 days and produce in average 61 offspring (Fucheng & Zhang 1990).

*Trichogramma pintoi* seems to have great potential as a biological control agent against the cotton bollworm, *H. armigera* (Silva & Stouthamer 1999).

## **Hosts**

### **a) *Ephestia kuehniella* Zeller**

*Ephestia kuehniella*, the Mediterranean flour moth is a lepidopterous pest of stored grain and dried plants from the Pyralidae family. It is the commonest *Ephestia* found in North America, where cereals and flour are stored (Richardson 1926). This is also a host largely used for parasitoid mass rearing, because of the ease with which it is reared in small space and its resistance to diseases (Richardson 1926). Their eggs are good hosts for laboratory rearing of *Trichogramma*. In fact, when compared with

*Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae), it appears that *E. kuehniella* eggs are preferred by *Trichogramma* (Tabone et al. 1999). The eggs are beige, round (0.5x 0.3 x 0.3 mm), have a volume of approximately 0.28 mm<sup>3</sup> (Bai et al. 1992), and are reticulate (Salt 1935). Their size permits only one individual to develop.

## **Biological control**

Biological control is the “regulation by natural enemies of another organism’s population density at a lower average density that would otherwise occur” (DeBach 1974). The term “natural enemies” includes parasitoids, predators and pathogens. The DeBach’s definition does not necessarily imply any activity by man. Classical biological control is the importation, and establishment of exotic natural enemies to add a density-dependent mortality factor for a specific pest, generally exotic too (DeBach 1974). In inoculative biological control, a species is introduced early in the season to establish itself temporarily (Li 1994). It prevents pest populations from reaching the injury level. In inundative biological control, a large number of individuals are released for an immediate effect (Li 1994).

### **a) *With Trichogramma***

Over 32 million ha are treated each year in agriculture and forestry worldwide with *Trichogramma* species, generally lepidopterous pests, on corn, sugar cane, cotton, fruit trees and vegetables (Li 1994). The principal countries using them are the former USSR, China and Mexico with over two million ha treated every year (Li 1994). Only a small number of the large number of species of *Trichogramma* are used commercially: *T. dendrolimi* Matsumura, *T. evanescens*, *T. chilonis*, *T. japonicum* Ashmead, *T. pretiosum*, *T. maidis* Pinto & Voegelé and *T. ostrinae* Pang & Chen (Li 1994). It is important to select the species or strain most appropriate to the control of each pest (Li 1994). In addition, indigenous species are preferred to exotic species because the latter can eliminate indigenous species by competition (Li 1994).

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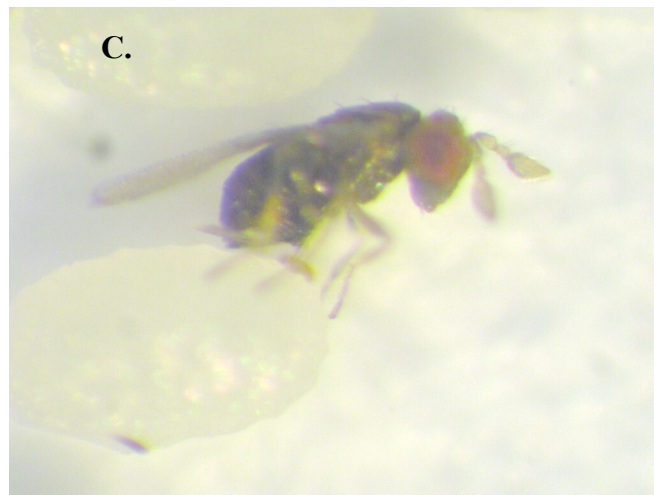
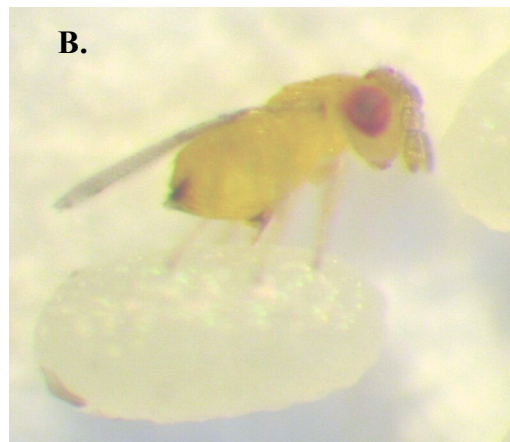
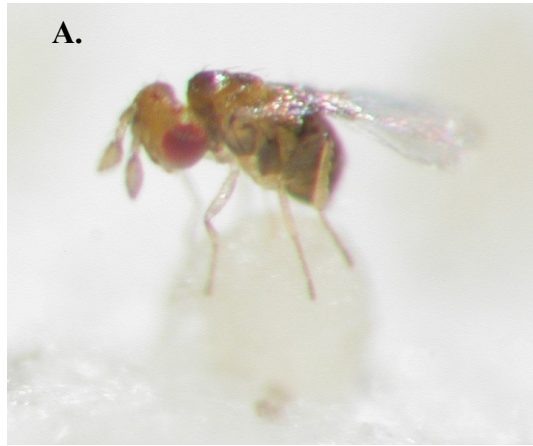
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**Figure 1.1:** *Trichogramma* females ovipositing in *Ephesia kuehniella* eggs. **A.** *T. evanescens*. **B.** *T. minutum*. **C.** *T. pintoi*.

## **CHAPTER II**

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# **IMPACT OF INTRA- AND INTERSPECIFIC COMPETITION ON SEX ALLOCATION BY *TRICHOGRAMMA* EGG PARASITOIDS**

## 1. ABSTRACT

Hymenopteran parasitoids change their sex ratios following different factors. One of these factors is the exploitation of a host patch by several females. The Local Mate Competition (LMC) states that when there are many foundresses on a patch, they should lay a higher sex ratio. The impact of both intra- and interspecific competition on sex allocation was measured in two egg parasitoids, *Trichogramma minutum* Riley and *T. pintoi* Voegelé (Hymenoptera: Trichogrammatidae), by comparing the sex ratio produced by females exploiting host patches alone and in groups. Results showed that the sex ratio produced by both species was higher when in groups, as predicted by the LMC model. When the sex ratio produced was compared between females either alone or in interspecific groups, *T. minutum* females deposited the same sex ratio, while *T. pintoi* produced more sons when in interspecific groups than when alone. These strategies may be related to the natural habitat and discrimination abilities of these species.

**Key words:** Local Mate Competition, interspecific competition, *Trichogramma*, Hymenoptera, egg parasitoids.



## 2. INTRODUCTION

Competition among individuals can be of two types: contest and scramble (Nicholson 1954). In contest competition, the winner gains everything he needs to survive and reproduce. In scramble competition, all population members have the same access to a resource and share it. The latter type is frequently found in animals where territoriality and social interactions are not well developed, as in insects (Price 1984).

In hymenopteran parasitoids, competition can take different forms: competition between females for hosts, competition between males for mates, and direct and indirect competition between larvae inside the same host (Dajoz 1974). However, for many parasitoid species, intense adult competition is unlikely to occur in natural habitat because hosts are highly dispersed and parasitoid density is low (Waage & Lane 1984). Nevertheless, in agro-ecosystems where parasitoids are mass-released, there is probably more adult competition (Waage & Lane 1984). Competition between adults could increase larval competition if there is superparasitism. Parasitoids generally avoid larval competition by discriminating between parasitized and unparasitized hosts and by laying eggs in unparasitized hosts (Werren 1980; van Alphen et al. 1987; van Dijken & Waage 1987; King 1993; Miura et al. 1994). Females discriminate hosts by detecting marking pheromones deposited by previous ovipositing females outside the host (Salt 1937; Rabb & Bradley 1970; Vinson & Guillot 1972; Bosque & Rabinovich 1979) or by internal marks or changes in the host (Jackson 1966; Fisher & Ganesalingam 1970; Greany & Oatman 1972; Chow & Mackauer 1986). When superparasitism occurs, the emerging clutch size and sex ratios can be different from what was laid by the first female.

The capacity to recognize that a host has already been parasitized by a different species is rare. It has been reported in *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae) (Wylie 1972), *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) (Wylie 1970), *Muscidifurax zaraptor* Kogan & Legner (Hymenoptera: Pteromalidae) (Wylie 1971), and *Anaphes victus* Huber [*Anaphes* n. sp.] (Hymenoptera: Mymaridae) (van Baaren et al. 1994; Huber et al. 1997). When multiparasitism occurs, the most competitive species generally wins the competition.

Females of these winning species can even search for previously parasitized hosts (and become occasional hyperparasites) to kill more rapidly the other species and then eliminate competition, like *Trichogramma dendrolimi* Matsumura when ovipositing with *T. evanescens* Westwood in laboratory (Pintureau et al. 1980), and *Eupelmus vuilleti* Crawford (Hymenoptera: Eupelmidae) with *Dinarmus basalis* Rondani (Hymenoptera: Pteromalidae) (van Alebeek et al. 1993).

After emergence, mate competition occurs among adults. Females of hymenopteran parasitoids ovipositing on a patch adjust their sex ratio to balance the level of mate competition. Female parasitoids can adjust the sex ratio they deposit because most hymenopteran parasitoids reproduce by arrhenotokous parthenogenesis: fertilized eggs become females and unfertilized ones become males. The Local Mate Competition (LMC) model predicts that when there is intraspecific competition (when there is more than one foundress on the same patch), each female should deposit a greater proportion of sons (a higher sex ratio), increasing competition for mating among males (Hamilton 1967). Depositing more males in these conditions increases the mother's fitness in two ways. First, if there are more males in a female progeny, there is a greater probability that sibmating will occur, and sibmating reduces the proportion of stranger genes shared by daughters. Secondly, a mother's son can mate with other females' daughters, therefore increasing the dispersion of the mother's genes. LMC has been demonstrated in numerous species (King 1987, 1996; van Welzen & Waage 1987; reviewed by Herre et al. 1997), both for simultaneous and sequential visits to a patch by different females (van Welzen & Waage 1987). Females can perceive the number of foundresses on a patch by the number of visual and physical contacts, the rate of encounter of parasitized hosts versus her parasitism rate, and the presence and concentration of chemical markers on the hosts (van Welzen & Waage 1987).

In contrast, the presence of interspecific competition should not result in a change in sex allocation, as the progeny of a given species should not compete for mate with the progeny of another species. The experimental results, however, are contradictory. When *Nasonia vitripennis* and *Spalangia cameroni* exploit a patch together, no sex allocation modification is observed, as predicted (King 1996), but

*Muscidifurax raptor* Girault & Saunders (Hymenoptera: Pteromalidae) females lay a higher sex ratio when placed on a patch with *Spalangia cameroni* than when alone (King & Seidl 1993). Another study found that the sex ratio produced by females increases with parasitoid density in both intraspecific competition (*Anisopteromalus calandrae* Howard (Hymenoptera: Pteromalidae)) and interspecific competition (*A. calandrae* and *Choetospila elegans* Westwood (Hymenoptera: Pteromalidae)) (Wen et al. 1994). However, as hosts are parasitized as the patch is exploited, it is not clear if positive results are a consequence of LMC or of the Host Quality model, that predicts a higher sex ratio when host quality is poor (Charnov et al. 1981). Host quality determines several individual parasitoid characteristics that influence fitness (size, fecundity, longevity, etc.) in both males and females (Charnov et al. 1981). However, females gain more fitness from developing in a good host than males do (van den Assem 1971; Werren & Simbolotti 1989; Ueno 1999). Females are then generally laid in higher quality hosts and males in lower quality hosts (van den Assem 1971).

In this study, I examined the effect of multiple foundresses on sex allocation in two egg parasitoids from the Trichogrammatidae family: *Trichogramma minutum* Riley and *T. pintoi* Voegelé. The sex allocation of these two species was measured both in intraspecific competition (LMC model) and in interspecific competition (when females of different species are on the same host patch). The hypothesis tested was that both species should follow the LMC theory (lay more sons when females are in groups), but that the sex ratio (proportion of males) should not be influenced by interspecific competition.

### **3. MATERIALS AND METHODS**

#### **General methods**

The *T. minutum* used in this study originated from Ontario and the *T. pintoi* from China. The rearings were maintained at 23±2°C, 50% RH and 16L:8D on eggs of the Mediterranean flour moth, *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae). The volume of these eggs is 0.28mm<sup>3</sup> (Bai et al. 1992) and permits only one

*Trichogramma* to develop. Cold-killed *E. kuehniella* eggs were glued on strips made of 3M Post-it® message pads of different sizes for different egg quantities: 2 mm x 4 mm for 50 eggs, 10 mm x 4 mm for 250 eggs and 10 mm x 8 mm for 500 eggs. Females used in the tests were taken from the rearing and were unfed, randomly mated, with no oviposition experience and aged under 24 hours.

### **Presence of virgin females in rearing**

The proportion of virgin females in the rearing was measured for each species to evaluate the possible bias due to the presence of virgin females ovipositing on the patches. Thirty-four *T. minutum*, and 33 *T. pintoi* females were randomly chosen from the rearing and individually placed in a vial with a patch of 250 host eggs. The vial was frozen after 14 days, a delay sufficient to permit to all individuals to emerge. The males and females were counted. Females producing only male progeny were considered as virgin.

### **Interspecific mating**

*Trichogramma minutum* and *T. pintoi* were chosen because they can be distinguished by the color of their body; yellow for *T. minutum* and black for *T. pintoi*. They are considered as two valid species (Pinto 1998) but their capacity to intercross was verified. A *T. pintoi* female and a *T. minutum* male were placed in a vial with 250 host eggs. The vial was frozen after 14 days, and the progeny produced was counted and sexed (36 replicates). The inverse procedure was also conducted (33 replicates). This information was necessary to validate my hypothesis that females experiencing interspecific competition should not change their sex allocation as no competition for mates would occur between their progeny. The presence of male-only progeny would confirm that these two species are not cross fertile.

### **Intraspecific competition**

The impact of intraspecific competition between females on sex allocation for each species was measured. In all tests, enough host eggs were provided for the number of progeny produced by a female in one day: 50 eggs for a female alone and

500 eggs for groups of females. The number of host eggs available per female was constant to eliminate the effect of host availability. Females were kept in a vial with host eggs for 24h, to eliminate the longevity factor. Vials were frozen after 14 days, and the progeny produced was counted and sexed.

In a first test, the sex ratio of females alone was determined. For each species, a female was isolated in a vial with host eggs. The number of replicates was 48 for *T. minutum*, and 32 for *T. pintoii*.

In a second test, the sex ratio produced by females in intraspecific groups was examined. For each species, ten females were placed in a vial with host eggs. The group size (ten females) was chosen to have enough effect to be easily detectable. The number of replicates was 31 for *T. minutum*, and 38 for *T. pintoii*.

### **Interspecific competition**

The impact of interspecific competition on the sex allocation deposited by the females of each species was evaluated. In both tests, enough host eggs were provided for the number of progeny produced by a female in one day: 50 eggs for females alone and 500 eggs for groups of females. The number of host eggs available per female was constant, to eliminate the effect of host availability. Females were kept in a vial with host eggs for 24h, to eliminate the longevity factor. Vials were frozen after 14 days, and the progeny produced was counted and sexed.

In a first test, the sex ratio of females alone was determined. For each species, a female was isolated in a vial with host eggs. The number of replicates was 31 for *T. minutum*, and 37 for *T. pintoii*.

In a second test, the sex ratio produced by females in interspecific groups was examined. One *T. pintoii* female and nine *T. minutum* females were placed in a vial with host eggs (30 replicates). The inverse procedures (one *T. minutum* female with nine *T. pintoii* females) were also conducted (32 replicates).

## **Statistical analyses**

To compare sex ratios, a 2x2 contingency table was done and a Chi-square was calculated. For offspring number comparisons, normality was tested with a Shapiro-Wilk test. As some data were not normal, a Wilcoxon test was conducted.

## **4. RESULTS**

### **Presence of virgin females in rearing**

For both *Trichogramma* species, all females taken from the rearing were mated as all females that oviposited produced females.

### **Interspecific mating**

When female *T. minutum* and male *T. pintoi* were placed together, all females that oviposited produced males only, an indication that these females were not fecundated. Three females (out of 33) laid no progeny. When female *T. pintoi* were placed with male *T. minutum*, the females also produced only males except for three females (out of 36) that produced no progeny.

### **Intraspecific competition**

Both *T. minutum* and *T. pintoi* females laid a significantly higher sex ratio in intraspecific groups than alone (Table 2.1). Females of both species also produced fewer offspring in groups than alone (Table 2.3).

### **Interspecific competition**

When the two species exploited a patch at the same time, *T. minutum* females deposited the same sex ratio as when they were alone (Table 2.2), and their number of offspring was also equal in both situations (Table 2.3). *T. pintoi* produced a higher sex ratio in interspecific group than alone (Table 2.2), and produced less offspring when in interspecific group than alone (Table 2.3).

## 5. DISCUSSION

The test on proportion of virgin females in rearing confirmed that 100% of female *T. minutum* and *T. pintoii* in my rearing were mated. This confirms that, in rearing conditions where females cannot disperse, the number of males is enough to allow them to mate all females. I therefore consider that there was no bias toward higher sex ratio due to virginity in my results.

Females in intraspecific groups laid a significantly higher sex ratio than females alone, for both *T. minutum* and *T. pintoii*. This confirmed my hypothesis based on the Hamilton's LMC theory (1967); females in groups perceived a potential competition for the mating of their sons, and deposited a higher proportion of males to increase their own fitness. In high competition situations, a difference in the sex ratio produced could also be explained by differential mortality between males and females, but such mortality is significant only when superparasitism is important (Schmidt 1994). In my experiment, the number of hosts available per female was kept constant, and was sufficient for the females to have access to unparasitized hosts, so superparasitism is assumed to be negligible (Godin & Boivin 2000).

There are two possible mechanisms to explain the higher sex ratio produced by females under situations of local mate competition: interference (Waage 1982) and change of sequence in oviposition (van Welzen & Waage 1987). When interference acts, females in groups lay fewer offspring than when alone. When males are laid first in the oviposition sequence and fewer eggs are laid, the produced sex ratio is thus higher. In cases where a change in the sequence of males and females deposited is involved, females modify the proportion of males laid at the beginning of a sequence as intraspecific competition increases. In this experiment, the sequence of oviposition has not been examined, but the total number of offspring laid per female of both species was lower for females in groups than for single females, as predicted by the interference mechanism. Several *Trichogramma* species, such as *T. evanescens*, *T. chilonis* Ishii, *T. brassicae* Bezdenko, and *T. pretiosum* Riley show a precise sex ratio strategy while exploiting host patches (Flanders 1935; Suzuki et al. 1984; Wajnberg 1993; Boivin, unpublished data.). This oviposition strategy increases the probability of laying sons in the first or second oviposition. When females using such a strategy

lay smaller clutch sizes on a patch because of increased competition, the total sex ratio produced is expected to be higher. As the intensity of the competition increase, the intensity of the impact on sex allocation predicted by the LMC theory should also increase.

The sex ratios obtained in these experiments were compared with the theoretical model of Hamilton, modified for haplodiploid species. The model predicts that the sex ratio produced by haplodiploid species should be  $r = (N-1)(2N-1)/N(4N-1)$ , where  $r$  is the sex ratio and  $N$  is the number of foundresses (Taylor & Bulmer 1980). For both species, the sex ratios obtained for females in groups were lower than predicted (Table 2.1). In nature, depending on the mating structure, some daughters could have dispersed still virgin and mate outside of patch. A lower proportion of sons than predicted by the model could then be sufficient for males to compete for, and mate with, the remaining females. This non-respect of the local mating assumption of Hamilton would explain the lower sex ratio in my results. Such off-patch mating seems to be significant in some *Trichogramma* species (Kazmer & Luck 1991; Antolin 1999).

The test conducted to evaluate interspecific mating showed that *T. minutum* and *T. pintoii* are two valid species. However, when this experiment was done, mating was observed between the two species, even if no offspring was produced. Interspecific mating, without fecundation, has been observed in other *Trichogramma* species (Pintureau 1991). Such interspecific mating implies costs for the females who mate without being able to fertilize eggs. Either these females are unable to discriminate the male species, or the costs may be higher to refuse interspecific males than to accept them.

No significant difference was found between the sex ratio produced by female *T. minutum* alone and in interspecific group, while *T. pintoii* females laid a higher sex ratio in interspecific group than alone. This suggests that female *T. minutum* recognized the *T. pintoii* females as a different species and did not perceive the progeny of *T. pintoii* females as potential mating competition for their own progeny.

In opposition to *T. minutum*, *T. pintoii* females could be unable to recognize *T. minutum* females as a different species. The capacity of interspecific discrimination



could be related to the capacity of a species to assess the environment. Interspecific discrimination is functional in sympatric species (Vet et al. 1984; Pijls et al. 1995; van Baaren et al. 1994), so these species should have better interspecific discrimination ability. My results then suggest that *T. minutum* is in sympatry with other *Trichogramma* species. Information on *T. minutum* from Ontario is relatively easy to obtain, and other *Trichogramma* species are found in the same kind of habitat and on the same hosts and host plants (Pinto 1998). Results also suggest that *T. pintoii* is not in sympatry with other *Trichogramma* species in its natural habitat. However, information on *T. pintoii* is more difficult to obtain because the strain is not from North America but from China, and is less documented.

A second explanation for this change in sex ratio by *T. pintoii* females could be larval competition. In multiparasitism, one species may be stronger and win the competition. As *E. kuehniella* eggs can sustain the development of only one *Trichogramma*, larval competition leading to the survival of only one individual occurs. When it occurs, a part of the progeny of the weaker species is killed before emergence. This mortality can have an impact on the emerging sex ratio depending on the proportion of each sex killed by the other species. To evaluate this possibility, the numbers of offspring emerging for *T. minutum* and *T. pintoii* in interspecific groups have been compared with the number of offspring of females in intraspecific groups (Table 2.3). My results showed that *T. minutum* produced the same number of progeny in intra- and interspecific groups, indicating no significant effect of multiparasitism for this species. For *T. pintoii*, the offspring produced by females in both situations was not the same, and more offspring emerged in intraspecific groups than in interspecific groups. If mortality affected more females than males, then the higher sex ratio of *T. pintoii* could be explained by multiparasitism rather than by sex ratio adjustment by females. Therefore, there may be a bias in results for *T. pintoii*. In addition, *T. minutum* has a shorter development time than *T. pintoii* (approximately two days earlier), and a study on interspecific competition with *Apoanagyrus lopezi* De Santis and *A. diversicornis* Howard (Hymenoptera: Encyrtidae) suggests that the species with shortest development time emerges more often (Pijls et al. 1995). In fact,

a species developing faster, like *T. minutum*, could use all resources and then indirectly kill larvae of the other species, here *T. pinto*.

Interspecific competition can have an important impact in biological control programs where more than one parasitoid is introduced or an indigenous species is already present in field. Some authors argue that it is helpful (Smith 1929; DeBach 1966; Huffaker et al. 1976), while some argue that it is not (Pemberton & Willard 1918; Turnbull & Chant 1961; Kakehashi et al. 1984; Briggs 1993; Rosenheim et al. 1995; Murdoch et al. 1998). The effect of competition seems to depend on the type of interactions occurring; lethal interference competition, like multiparasitism or facultative hyperparasitism (Briggs 1993) is supposed to be disruptive for biological control (Collier et al. 2002). In fact, competition between two species can have different effects on both species. It could reduce the population of one species (Voegelé et al. 1979; Pintureau et al. 1980) or modify the population sex ratio produced by females as demonstrated in this study.

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**Table 2.1:** Sex ratios produced by *T. minutum* and *T. pintoii* females alone and in intraspecific groups.

	<i>Trichogramma minutum</i>		<i>Trichogramma pintoii</i>	
	Alone	In group	Alone	In group
Hamiltonian sex ratio	minimal*	0.439x	minimal*	0.439x
Obtained sex ratio (proportion of males)	0.230a	0.415by	0.260a	0.317by
Number of broods (n)	48	31	32	38

Hamiltonian sex ratio is obtained with the equation  $r=(N-1)(2N-1)/N(4N-1)$  (Taylor & Bulmer 1980). Within a species, letters a-b denote a significant difference between sex ratios alone and in groups (in rows), while letters x-y denote a significant difference between sex ratios in group (in columns) (Chi-square test,  $P \leq 0.05$ ).

\* The minimal sex ratio indicates that females lay only enough sons to fertilize all their daughters.

**Table 2.2:** Sex ratios produced by *T. minutum* and *T. pintoii* females alone and in interspecific groups.

	<i>Trichogramma minutum</i>		<i>Trichogramma pintoii</i>	
	Alone	In group	Alone	In group
Sex ratio (proportion of males)	0.240 <sup>a</sup>	0.267 <sup>a</sup>	0.244 <sup>a</sup>	0.338 <sup>b</sup>
Number of broods (n)	31	32	37	30

Within a species, different letters indicate significant differences in rows (Chi-square test,  $P \leq 0.05$ ).

**Table 2.3:** Number of offspring produced by *T. minutum* and *T. pintoï* females alone and in intra- and interspecific groups.

		<i>Trichogramma minutum</i>		<i>Trichogramma pintoï</i>	
		Alone	In group	Alone	In group
Offspring ± s.e.	Intraspecific competition	30.29 ± 0.12 $ax$	28.23 ± 0.56 $bx$	30.25 ± 0.70 $ax$	28.15 ± 0.50 $bx$
	Interspecific competition	29.58 ± 1.40 $ax$	30.28 ± 1.43 $ax$	30.11 ± 0.95 $ax$	25.47 ± 0.90 $by$

Within a species, letters a-b denote a significant difference between offspring alone and in group (in rows), while letters x-y denote a significant difference between offspring in intra- and interspecific group (in columns) (Wilcoxon test,  $P \leq 0.05$ ).

## CONNECTING TEXT I

In the second chapter, local mate competition and its impact on sex allocation have been verified for *Trichogramma minutum* and *T. pintoi*. However, one of the important assumptions of LMC theory is that a population is very structured and all matings occur locally on the patch, before dispersion of females to find hosts. This assumption has not been verified for these species. The intent of the next chapter is then to look at the pre-mating dispersion and pattern of dispersion of *Trichogramma* species to evaluate the proportion of local and non-local mating.

Chapter to be submitted to: **Environmental Entomology**

## **CHAPTER III**

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**PRE-MATING DISPERSION IN THE EGG**

**PARASITOID *TRICHOGRAMMA***

## 1. ABSTRACT

Gregarious and quasi-gregarious parasitoid females can either mate on the emergence patch or disperse and mate later. The costs and benefits of each option depend on the genetic proximity of individuals at the emergence site and the probability of encountering a mate outside the patch. The temporal pattern of dispersion of males and females and the level of pre-dispersal mating for females was measured in three egg parasitoid species, *Trichogramma evanescens* Westwood, *T. minutum* Riley and *T. pintoi* Voegelé (Hymenoptera: Trichogrammatidae). Results showed that *T. minutum* has a higher proportion of virgin females dispersing than *T. pintoi* and *T. evanescens*. However, the fact that a proportion of dispersing virgin females was found in all three species indicates some level of genetic exchanges between sub-populations of these egg parasitoids. Results suggest that all species can show partial local mating at different levels.

**Key words** Pre-mating dispersion, virgin females, egg parasitoids, *Trichogramma*.

## 2. INTRODUCTION

Following emergence, hymenopteran parasitoid females can either mate locally before dispersing for host searching or disperse from the patch still virgin and find an off-patch mate. However, for solitary species where only one individual develops inside a host, females must find an off-patch mate by dispersing. Virginity is then predicted to be more common in solitary species, as no mates are present at emergence. Contrary to this prediction, the proportion of virgin females is higher in gregarious species than in solitary species (Godfray & Hardy 1993). In quasi-gregarious species, individuals develop alone inside a host but hosts are aggregated. The patch is then composed of a group of hosts rather than only one host, but as in gregarious species, mating can occur among individuals emerging from the patch. Proportion of virgin females should then be comparable to the one of gregarious species.

While mating on the patch increases the probability of mating, it also increases the probability of sibmating depending on the number of females that have colonized that patch. When females disperse before mating, the probability of sibmating decreases, but the risk of finding no mate and remaining virgin increases. These choices will depend on the importance of the costs of each choice for each species. In most parasitoid species, the probability of encountering a mate outside the patch is very low (Waage & Ming 1984), so the cost of pre-mating dispersion is high. In addition, in species where inbreeding depression is absent or at a low level, the cost of on-patch mating is lower. On-patch mating is then expected to be the rule in such species, like the haplodiploid arrhenotokous species, where males come from unfertilized eggs and females from fertilized eggs. In these species, almost no inbreeding depression has been shown (Brückner 1978; Antolin 1999; Werren 1993).

One factor that can increase on-patch mating for these species without inbreeding depression, is synchronous or overlapped emergences between males and females (Pompanon et al. 1995). Males frequently emerge before females to maximize the number of females they mate (Doyon & Boivin, unpublished data), and protandry is generally the rule in quasi-gregarious and gregarious species

(Doyon & Boivin, unpublished data; Pompanon et al. 1995; Hirose et al. 1988; Hébert et al. 1989). However, even if inbreeding depression is important, emergence can be synchronous and on-patch mating can be the rule if females are capable of kin discrimination, like in *Bracon hebetor* (Say) (Hymenoptera: Braconidae) (Ode et al. 1995).

Because of their low dispersion capacity and their short longevity, many parasitoids, such as *Trichogramma*, a quasi-gregarious egg parasitoid, are generally considered as mating locally. It is one of the assumptions of local mate competition (LMC) theory (Hamilton 1967), which states that when there is more than one foundress on a patch, they will each lay more sons to increase competition among them and increase their own fitness. This theory has been demonstrated in many species (King 1987, 1996; van Welzen & Waage 1987; reviewed by Herre et al. 1997; Martel & Boivin, unpublished data), assuming that mating is fully local. However, some authors have suggested, on the basis of indirect evidence, that partial local mating is more frequent than expected (Ikawa et al. 1993; Hardy 1994).

Although mating on the natal site is common in haplodiploid arrhenotokous species, virgin females are found in nature (Godfray & Hardy 1993). The presence of virgin females in a population has important consequences in haplodiploid parasitoids. Virgin females can lay only male clutches and therefore increase the population sex ratio. Such a constrained sex ratio is especially disadvantageous in species with local mate competition because virgin females are unable to produce the favored sex ratio, which is female-biased (Godfray 1990; Hardy & Godfray 1990; Godfray & Hardy 1993). Pre-mating dispersion can be a cause of virginity if females are unable to find an off-patch mate. However, virginity could also be the consequence of insufficient males on a patch, differential mortality (Green et al 1982; Nagelkerke & Hardy 1994), and limited insemination capacity of males (Sekhar 1957; Schlinger & Hall 1960; Wilson 1961; Gordh & DeBach 1976; Nadel & Luck 1985; Godfray 1990). A way to prevent these causes is to lay more males (Heimpel 1994), or have a precise sex allocation, when males are laid early in the oviposition sequence,



assuring at least one male for each clutch (Flanders 1935; Suzuki et al. 1984; Wajnberg 1993; Boivin, unpublished data). Although the costs of virginity if no off-patch mating occurs are high, the benefits obtained from outbreeding are important for the individuals. In addition, it allows genetic exchange between sub-populations.

In this study, the proportion of virgin females leaving a patch was examined under laboratory conditions in *Trichogramma evanescens* Westwood, *T. minutum* Riley and *T. pintoi* Voegelé. The hypothesis tested was that a low proportion of unmated females should be dispersing to find a non-related mate and, in consequence, assure some genetic exchanges between sub-populations. However, this proportion should be low because of the low probability of finding a mate outside the natal patch for females (Waage & Ming 1984). The *Trichogramma* species tested belong to different groups: *T. pintoi* is in the Parkeri section and *T. evanescens* and *T. minutum* are in the Exiguum section, in two different sub-groups, respectively the *evanescens* and *minutum* groups. (Pinto 1998). These species were chosen to cover a variety of *Trichogramma* variability. The temporal pattern of dispersion for females (mated and virgins) and males was also examined for the same three species. The tested hypothesis is that males should disperse later to mate with as many females as possible, and that unmated females should disperse later than mated females to increase their probability of being mated locally, and disperse only if there is no possibility for them to mate.

### **3. MATERIALS AND METHODS**

#### **General Methods**

The *T. minutum* used in this study originated from Ontario, the *T. pintoi* from China, and the *T. evanescens* from Egypt. The rearings were maintained at  $23\pm 2^{\circ}\text{C}$ , 50% RH and 16L:8D on eggs of the Mediterranean flour moth, *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae). The volume of these eggs is  $0.28\text{mm}^3$  (Bai et al. 1992) and permits only one *Trichogramma* to develop. Cold-killed *E. kuehniella* eggs were glued on strips made of 3M Post-it® message pads of 10

mm x 4 mm containing 250 eggs for oviposition of one female. All parasitized host eggs used in the experiment were taken from the rearing.

### **Mating structure and pattern of dispersion**

The proportion of virgin females in the dispersing population was measured for *T. evanescens*, *T. minutum* and *T. pintoï*. For each species, a patch of 350 parasitized hosts was placed in the center of a 1 m<sup>3</sup> cage made of white fabric, on a wood plate placed at 9.5 cm above the cage's floor. The host patch was placed above the floor to incite the *Trichogramma* adults to fly, instead of just walking, away from the patch when dispersing. Individuals landing on the cage's floor or walls were considered as having left the patch. To describe the temporal pattern of dispersion of both males and females *Trichogramma*, every 15 minutes, during 4.5 hours, the *Trichogramma* adults present on the floor or walls of the cage were collected individually in vials and sexed. This experiment was repeated five times for each species.

To measure the proportion of virgin females in the dispersing population, females were individually placed in a vial with a patch of 250 host eggs. These vials were frozen after 13-14 days, a delay sufficient to permit all individuals to emerge. The absence of females in the progeny was used to determine the proportion of virgin females. Proportion of virgin females was compared between species using contingency tables and Chi-square.

## **4. RESULTS**

### **Pre-mating dispersion**

The proportion of virgin females in the dispersing population was significantly higher in *T. minutum* than in *T. evanescens* and *T. pintoï* (Table 3.1). The proportion of males in the dispersing population in *T. pintoï* is higher than in the two other species (Table 3.1).

## **Temporal dispersion**

In *T. evanescens*, *T. minutum*, and *T. pintoi* individuals started to disperse around 30-40 minutes after light onset (Figure 3.1A, B, C). For *T. evanescens* and *T. minutum*, an important peak in mated females dispersal was around 75 minutes. In *T. pintoi*, a similar peak was also found around 75 minutes, but dispersion was distributed more equally during the experiment (Figure 3.1).

In *T. evanescens* and *T. minutum*, most of the few males dispersing did so after the peak of females' dispersion. In *T. pintoi*, more males dispersed and their pattern of dispersion was similar to mated females; dispersion started around 60 minutes and continued throughout the experiment.

## **5. DISCUSSION**

### **Pre-mating dispersion**

In the three *Trichogramma* species, the proportion of virgin females in the dispersing population was low. The results thus suggest that these species can show partial local mating at different levels (Hardy 1994). This level is higher for *T. minutum* with 6.4% than in *T. evanescens* and *T. pintoi* where it is under 0.5%. The results then point to some level of genetic exchange between sub-populations. However, as proportion of virgin females in the dispersing population was low for the three species, their population is relatively structured and most mating occurs within the emergence patch.

Parasitized eggs used were taken from a rearing where there is high competition level between females. These females were then laying a higher sex ratio (more males) than in nature where the competition level is low. As more sons were emerging on the patch, daughters had more mating opportunities and the probability that virgin females dispersed was lower than in nature. My results are then probably different than what happens in natural habitat. However, as released eggs in biological control are generally parasitized in the same condition as in my experiment (in mass-rearing), these results are probably closer to the ones obtained following biological control release than in nature.

Another important factor to consider is the time-limited aspect of the experiment. As time passed, males could become sperm-depleted and more females could disperse still virgin. A longer experiment would then probably show different proportion of virgin females in the dispersing population. In addition, approximately 13%-27% of individuals dispersed within the 4.5 hours of the experiment, so there is more than 70% of individuals for which dispersal was not observed.

### **Temporal dispersion**

In the three *Trichogramma* species, the few virgin females that dispersed did so at the peak of dispersion of mated females. During that period most males were mating and the females that were unable to obtain males at that point probably decided to leave the patch. Almost all virgin females dispersed in the first half of the experiment although I predicted that virgin females should have dispersed later than mated females. In *Trichogramma*, mating enhances the propensity to fly and disperse, and virgin females fly later (Forsse et al. 1992; Pompanon et al. 1995). However, virgin females in this study, even when dispersing relatively early, were not the first individuals to do so.

Finally, males *T. evanescens* and *T. minutum* dispersed mainly after the dispersal peak for mated females, when less females were present on the patch. The propensity to fly for males is lower than females' in *Trichogramma*, and males tend to stay longer when many females are present on the patch (Forsse et al. 1992). Contrarily to *T. evanescens* and *T. minutum*, a high number of male *T. pintoi* dispersed. Their dispersion pattern was similar to that of mated females; an increase 60 minutes after the onset of light, then a regular dispersion until the end of the observation period. Males of this species started dispersing early in the experiments when few females were present. As the proportion of males dispersing is high, these males can mate with emerging females of other patches (Nunney & Luck 1988), and it could increase the off-patch mating level. However, this high proportion of males could be linked to a higher sex ratio in the population, but this factor has not been measured.

Observing the dispersion pattern of these three species give us cues about the mating strategy of *Trichogramma* species. All species have the potential of off-patch mating, *T. minutum* having the higher one. Therefore, these three species are probably under different levels of partial local mating. These results could explain some divergent results obtained previously. Previous experiments showed that *T. minutum* and *T. pintoii* follow the LMC theory (Martel & Boivin, unpublished data), but the obtained sex ratios were different from the ones predicted for haplodiploid species (Taylor & Bulmer 1980). As local mating is an important assumption of the LMC theory, the non fully local mating observed in the present study could explain the divergence obtained previously.

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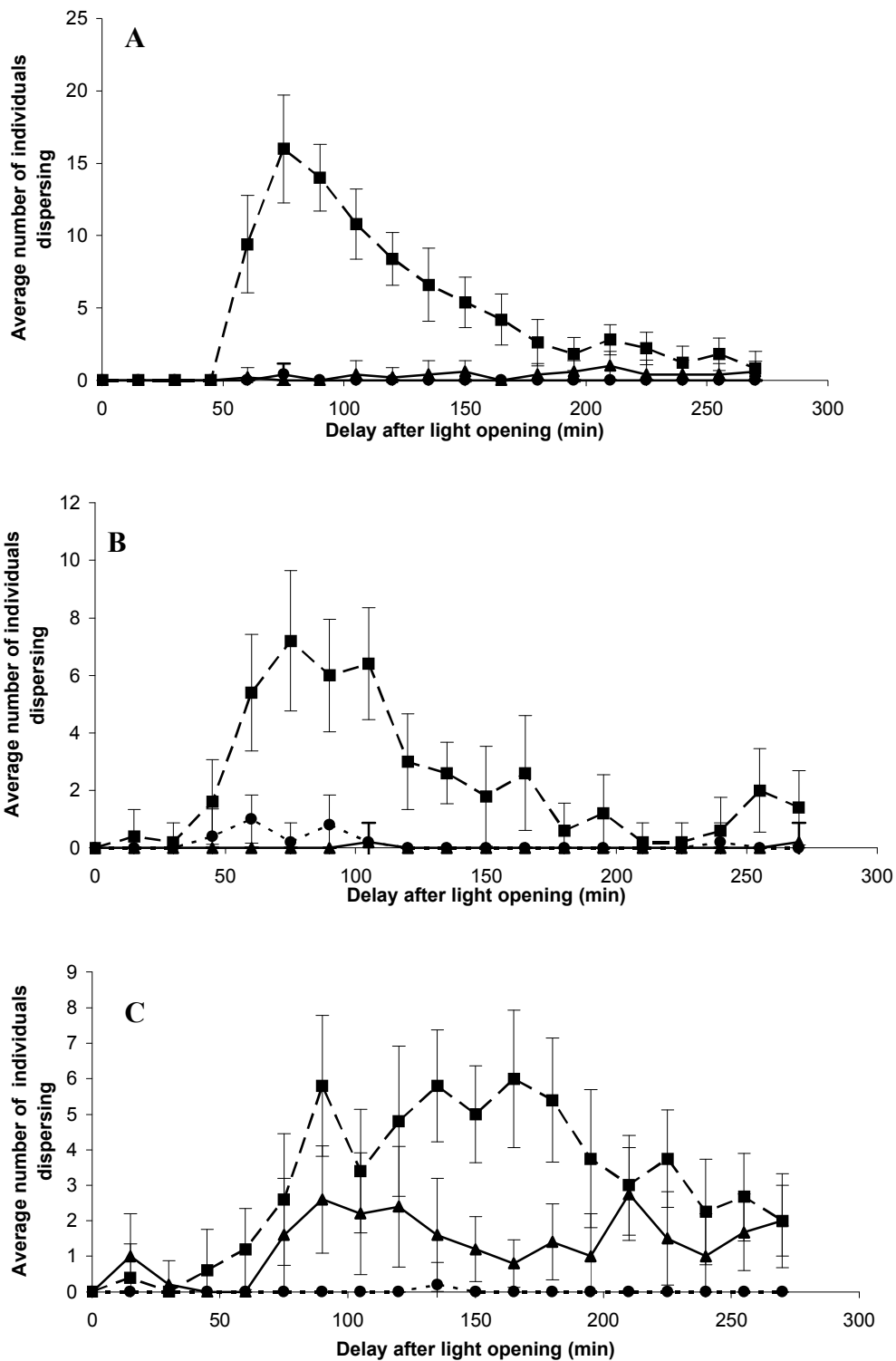


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**Table 3.1:** Proportion of females (virgin and mated) and males of *Trichogramma evanescens*, *T. minutum* and *T. pintoi* in the dispersing population in a cage for 4.5 hours.

<b>Proportion (%) of</b>		<b><i>T. evanescens</i></b>	<b><i>T. minutum</i></b>	<b><i>T. pintoi</i></b>
Females	Virgin	0.20 <sup>a</sup>	6.42 <sup>b</sup>	0.49 <sup>a</sup>
	Mated	93.66	92.77	72.35
Males		6.14 <sup>a</sup>	0.81 <sup>a</sup>	27.16 <sup>b</sup>
Total number of individuals dispersing (N)		470	233	382

Different letters in a row indicate significant differences (Chi-square test,  $P \leq 0.05$ ).



**Figures 3.1:** Temporal dispersion in cage of virgin females (circles), mated females (squares), and males (triangles) of **A.** *T. evanescens*. **B.** *T. minutum*. **C.** *T. pintoi*.

## CONNECTING TEXT II

Pre-mating dispersion and temporal pattern of dispersion have been observed for *Trichogramma minutum*, *T. pintoi*, and *T. evanescens* in the preceding chapter. It appears that mating occurs mainly locally for the three species, but the proportion of virgin females dispersing is the lowest in *T. evanescens*. However, for *T. minutum*, in opposition to the two other species, there is a higher proportion of virgin females dispersing, allowing a small number of off-patch mating. A higher proportion of males also dispersed in *T. pintoi*, allowing off-patch mating.

In the next chapter, the impact of inbreeding on sex allocation will be examined for *T. evanescens*, which has a very structured population where mating occurs locally, in which inbreeding is supposed to happen frequently.

Chapter submitted to: **Biocontrol Science and Technology**

## **CHAPTER IV**

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**IMPACT OF INBREEDING ON SEX ALLOCATION**

**BY *TRICHOGRAMMA EVANESCENS***

## 1. ABSTRACT

Many hymenopterous parasitoids have structured populations; mating occurs locally at emergence, between progeny of the patch foundresses. In this situation, inbreeding is more likely to occur than in panmictic populations. For many of these species however, there is no inbreeding depression. Because of the genetic structure of arrhenotokous species, where males are haploid and females are diploid, there is an asymmetry in relatedness between the mother and her sons and daughters following inbreeding. Under this asymmetry, daughters become more valuable and sex ratio is then expected to be more female-biased. This theory has been tested in an egg parasitoid, *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae). Results suggest no impact of inbreeding on sex allocation. However, as females were in a condition of high local mate competition, I expect them to adjust their sex ratio following mostly local mate competition rather than inbreeding in this experiment.

**Key words:** Inbreeding, outbreeding, sex ratio, *Trichogramma*, egg parasitoids.

## 2. INTRODUCTION

Sibmating generates, in the vast majority of species, a decrease in progeny fitness which is called inbreeding depression. Because of this depression, many species have developed some inbreeding avoidance behaviours (Ralls et al. 1986; Pusey 1987; Blouin and Blouin 1988; King and King 1995) including kin recognition (Wu et al. 1980; Bateson 1982; Kareem and Barnard 1982; Hepper 1983) and dispersal (Pusey 1980; Pusey and Packer 1987). However, in haplodiploid species, where males are haploid and females are diploid, sibmating causes generally no, or only a low level, of inbreeding depression (Brückner 1978; Antolin 1999; Werren 1993). The Hymenoptera parasitoids are haplodiploid species that reproduce mostly by arrhenotokous parthenogenesis; males come from unfertilized eggs and females come from fertilized eggs. Species of hymenopteran parasitoids such as *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae) (King and King 1995), *Cothonaspis boulardi* (Hymenoptera: Eucoilidae) (Biémont and Bouletreau 1980), and *Dinarmus vagabundus* (Timberlake) (Hymenoptera: Pteromalidae) (Rojas-Rousse et al. 1988), show no inbreeding depression following sibmating. Contrarily to diploid species, deleterious recessive alleles are directly exposed to natural selection in haploid males of haplodiploid species (King et al. 1969; King and King 1995) while female-limited traits, like fecundity, could still be subjected to inbreeding depression (King and King 1995).

In gregarious and quasi-gregarious (when development is solitary but hosts are aggregated) hymenopteran parasitoid species, inbreeding is likely to occur when mating takes place locally on emergence site. The probability of sibmating increases as the number of females that oviposit on a given host patch decreases (Frank 1985). Inbreeding is generally considered as the rule in haplodiploid parasitoid species (Hamilton 1967; Askew 1968); it is common in *Trissolcus basalis* (Wollaston) (Hymenoptera; Scelionidae) (Wilson 1961), where 81.3% of females mated locally shortly after emergence (Loch and Walter 2002), and in *Trichogramma* species with 55-64% of sibmating (Kazmer and Luck 1991).

The genetic structure of haplodiploidy induces an asymmetry in the relatedness of a mother with its progeny. Each individual receives a copy of its mother's genes, but only daughters receive a copy from their father. So both daughters and sons share 50% of the mother's genome, while daughters have 100% of the genes of their haploid father. Therefore, if a female and a male share common genes (as in inbreeding), the relatedness of the daughters to their mother will be more than 50% while the relatedness of sons with their mother will remain at 50%. Because of this asymmetry, daughters become more valuable to their mother under sibmating. The sex ratio (proportion of males) produced under sibmating is then expected to be more female-biased (Greeff 1996). In haplodiploid pollinator fig wasps, species with a high level of inbreeding do have a lower sex ratio (Herre et al. 1997) but in *Leptopilina heterotoma* (Thomson) (Hymenoptera: Eucoilidae) (Hey and Gargiulo 1985), and *Spalangia cameroni* (King and King 1995), no effect of inbreeding on sex ratio has been observed.

In this study, I examined the effect of sibmating on sex allocation in the egg parasitoid *Trichogramma evanescens* Westwood. The sex allocation of this species was measured under both inbreeding and outbreeding. The hypothesis tested was that the sex ratio should be more female-biased under sibmating than under outbreeding.

### **3. METHODS**

#### **General methods**

The *T. evanescens* used in this study originated from Egypt. The rearing was maintained at  $23\pm 2^{\circ}\text{C}$ , 50% RH and 16L:8D on eggs of the Mediterranean flour moth, *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae). The volume of *E. kuehniella* eggs is  $0.28\text{ mm}^3$  (Bai et al. 1992) and permits only one *Trichogramma* to develop. Strips of 2.5 mm x 4 mm made of 3M Post-it® message pads on which 125 cold-killed *E. kuehniella* eggs were glued were offered as oviposition sites to females *Trichogramma*. This represents a sufficient number of eggs for lifetime progeny of a female *T. evanescens*. Females used in the tests were taken



from the rearing and were unfed, randomly mated, with no oviposition experience and aged under 24 hours.

### **Sex allocation of inbreeding females**

Individual females from the rearing were isolated in a vial (3.7ml) with host eggs. After 3-4 days, the parasitized (black) eggs were isolated in capsules to obtain virgin *Trichogramma* individuals. At emergence, 10 days later, a son and a daughter of the same brood were mated together. Pre-mating duration, defined as the delay between the moment of contact of the male and the female in the capsule and the end of the mating, was measured. The mated female was then isolated in a vial with 125 host eggs for 24h. The vial was frozen after 13 days, a delay sufficient to permit all individuals to emerge, and individuals were counted and sexed (36 replicates).

### **Sex allocation of outbreeding females**

Two rearings were used, to ensure a certain genetic distance between the mating partners. The experiments began four generations after separation of the rearings.

A randomly mated female from one rearing and a randomly mated female from the other rearing were isolated separately in a vial with host eggs. After 3-4 days, the parasitized eggs were isolated in capsules to obtain virgin *Trichogramma* individuals. At emergence, 10 days later, a male from one rearing and a female from the other rearing were placed together and the pre-mating duration was measured. The mated female was then isolated in a vial with 125 host eggs for 24h. The vial was frozen after 13 days, a delay sufficient to permit all individuals to emerge, and individuals were counted and sexed (40 replicates).

### **Statistical analyses**

To compare pre-mating duration and number of progeny per female, t-tests were conducted. For sex ratios, a 2x2 contingency table was done and a Chi-square was calculated.

#### 4. RESULTS AND DISCUSSION

My results show that there are no significant differences for pre-mating duration, number of progeny per female, and progeny sex ratio (Table 4.1) between inbred and outbred females in *T. evanescens*. These results infirm my hypothesis that inbred females should lay a more female-biased sex ratio than outbred females.

The absence of impact of inbreeding on sex ratios may be explained by several causes. The female *T. evanescens* may be unable to evaluate the genetic distance between themselves and their mate. Without such discrimination capacity, they could not modify the allocated sex ratio accordingly. Another possibility is that because brothers and sisters emerged separately, and mating took place in a separate vial, the female considered it was non-local mating. Non-local mating has been shown to have an important influence on sex ratios by increasing it (Hardy 1994) as observed here.

The genetic distance between the two mates can also have an effect on the response of the female. The two rearings used in these experiments originated from a single rearing, and could be genetically close, even if reared separately for several generations. However, even if the genetic distance between the two rearings was not large, I should have observed an intermediary situation in which the progeny sex ratio would have been different from inbreeding progeny sex ratio, but this is not the case. Therefore, the level of relatedness between the two mates may not be important as in *Bracon hebetor* (Say) (Hymenoptera: Braconidae), where females consider that all individuals emerging from different hosts are non-kin, whatever their genetic distance with them (Ode et al. 1995). The mechanism used, or even the possession of such a discrimination ability, is not known in *Trichogramma* species.

The hypothesis that sibmated females lay a more female-biased sex ratio (Greeff 1996) is a general hypothesis for haplodiploid hymenopterous parasitoids. Parasitoids of the genus *Trichogramma* have very structured populations, because females colonize host patch and both sons and daughters of the same female emerge in groups. As a result, inbreeding is common in these species, and local

mating seems to be the rule in *T. evanescens* (Martel and Boivin, unpublished data). Under such conditions, females are expected to adjust their sex ratio following mostly local mate competition (Hamilton 1967) without considering inbreeding. Under the experimental conditions (no competition from other females), the females are expected to deposit a low sex ratio (the minimum number of sons necessary to mate all their daughters). As mating occurs mainly locally, then the population is very structured and effect of inbreeding could have been almost eliminated. In fact, the cost of depositing fewer males would be higher than the gain of laying more closely related-females, because part of these females would stay virgin. Therefore, a change in sex allocation under sibmating would be detectable only under some level of local mate competition. Absence of positive results in the present study could then be the consequence of the absence of competition. This hypothesis may be the more plausible one and would be interesting to be tested.

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**Table 4.1:** Pre-mating duration, progeny by female and sex ratios produced by *T. evanescens* in inbreeding and outbreeding situations.

	<b>Inbreeding</b>	<b>Outbreeding</b>
Pre-mating duration (sec) Mean $\pm$ s.d.	1.11 $\pm$ 1.06 $a$	0.92 $\pm$ 1.17 $a$
Progeny by female Mean $\pm$ s.d.	32.28 $\pm$ 5.99 $a$	34.35 $\pm$ 7.76 $a$
Sex ratio	0.22 $a$	0.21 $a$
Number of broods (n)	36	40

Significant differences in rows are indicated by different letters (Wilcoxon test for pre-mating duration and progeny by female, and Chi-square test for sex ratios,  $P \leq 0.05$ ).

**GENERAL CONCLUSION**

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The objectives of this study were: (1) to evaluate the impact of intra- and interspecific competition on sex allocation by *Trichogramma minutum* and *T. pintoii*; (2) to determine the temporal dispersion pattern and the importance of pre-mating dispersion in *T. evanescens*, *T. minutum* and *T. pintoii*; (3) to evaluate the impact of inbreeding and outbreeding on sex allocation by *T. evanescens*.

These experiments were conducted for three *Trichogramma* species. The species were chosen because they belong to different subgroups within the genus (Pinto 1998). These three species could then cover a variety of *Trichogramma* variability. Even if it could be possible to generalize the results to the whole genus, one should be prudent not to take into account that these results are true for every *Trichogramma* species.

In the second chapter, we have observed that intraspecific competition influences sex allocation by *T. minutum* and *T. pintoii* by increasing the number of sons laid. These results confirmed my hypothesis that both species should follow the Local Mate Competition theory (Hamilton 1967). *Trichogramma minutum* also confirmed my hypothesis by not modifying its sex ratio in interspecific competition, while *T. pintoii* increased its sex ratio in this situation. This sex allocation modification can be explained by high multiparasitism level with *T. minutum* winning competition against *T. pintoii* more often, or by a lack of species discrimination by *T. pintoii*, caused by a low number of sympatric species in its natural habitat. However, there is a lack of information on their natural habitat in the literature. Understanding the impact of competition is important for mass rearing and biological control. In mass rearing, females are under high intraspecific competition, and this could explain some problems caused by the high proportion of males in rearing. In addition, in agro-ecosystems where parasitoids are mass-released, there is probably some adult competition within a species (Waage & Lane 1984).

In the third chapter, results shown that *T. evanescens*, *T. minutum* and *T. pintoii* seem to mate mostly locally. However, in all species, at least a few females dispersed still virgin, allowing some off-patch mating. Such mating, even if rare, is important by increasing the genetic exchanges between sub-populations of

*Trichogramma*. *Trichogramma evanescens*, *T. minutum* and *T. pintoi*, even if they have structured population, can show different levels of partial local mating. Interestingly, these results could explain the differences between obtained sex ratios and predicted sex ratios in the second chapter. As the assumption of fully local mating from the LMC theory is not fully respected for *T. minutum* and *T. pintoi*, it could slightly change the impact of competition on sex allocation. In fact, sex ratios obtained were lower than those predicted. If some daughters disperse from the patch to mate outside it, there is a need for less males than if they all mate locally. The sex ratios laid by females could then be lower, as obtained in chapter II.

Finally, in the fourth chapter, we observed that females mating with sibs did not modify their sex ratio, in contrast to my hypothesis. My hypothesis was that females should lay more daughters under sibmating because they are more valuable under these conditions (Greeff 1996). This divergence of my results can have several explanations. Firstly, females could be unable to evaluate the genetic proximity of their mates. For example, *Bracon hebetor* evaluate the genetic proximity of an individual following the host from which it emerges; for a female, an individual emerging from the same host or from a neighbour host will be genetically closer than an individual emerging from a host which is located far from her own emergence site (Ode et al. 1995). Secondly, the genetic distance between the rearings could be insufficient to show a difference in sex allocation between inbreeding and outbreeding. And thirdly, the females could just not modify their sex allocation even if discriminating between sibs and non-sibs. As *T. evanescens* has a structured population with local mating, the important factor for sex allocation could be LMC instead of inbreeding, and their sex ratio could be too highly female-biased to decrease the number of males further. In fact, laying more females implies a cost because even if these females are more valuable, if there are fewer males in the clutch, some of these daughters will stay virgin.

These results bring a better understanding of the sex allocation and dispersion of some *Trichogramma* species. It could help improve mass rearing

and biological control with these parasitoids. In mass rearing, as the level of competition is very high, and there is a lot of females in a restraint space, the proportion of males will be high. Such a higher sex ratio could have a great impact in mass rearing and inundative release because it decreases the level of parasitism of the population. The releasing of more than one species, or of one species when there is some indigenous ones, could also decrease level of parasitism as some species react to interspecific competition by increasing their sex ratios, like *T.pintoi*. In classical and inoculative biological control where population establishes (DeBach 1974; Li 1994), the second generation would show low virgin females dispersing for the species studied because of the high sex ratio of individuals released. As most females ovipositing will be mated, there would be less males in the third generation of *Trichogramma* and the resulting population will have a higher parasitism level.

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