COMPETITION BETWEEN PARASITOIDS OF THE CABBAGE SEEDPOD WEEVIL: EFFECTS ON SEX RATIOS AND CONSEQUENCES FOR BIOLOGICAL CONTROL

by

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BSc. (Honours) University of Guelph 2007

THESIS
SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

In the
Department of Biological Sciences

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SIMON FRASER UNIVERSITY

Summer 2011

Approval

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Degree:		**Master of Science**
Title of Thesis:		**Competition between parasitoids of the cabbage seedpod weevil: effects on sex ratios and consequences for biological control**

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Abstract

In any biological control program, it is important to have an in-depth knowledge of the ecosystem. Competition between natural enemies can have unpredictable effects, potentially disrupting biological control of the pest in question. *Trichomalus perfectus* and *Mesopolobus morys* are two parasitoids of the cabbage seedpod weevil. Both are candidates for introduction as classical biological control agents in Canada. I examined the effects of intra- and interspecific parasitoid competition in field and laboratory experiments. I found that a multiple-species introduction would not be more effective at controlling pest populations than a single-species introduction. I also found *T. perfectus* produced a female-biased offspring sex ratio in response to intraspecific competition. Using a theoretical approach, I explored how this shift might affect parasitoid-host population dynamics and biological control, incorporating the influence of increased virginity in the parasitoid population.

Keywords: *Ceutorhynchus obstrictus*; *Trichomalus perfectus*; *Mesopolobus morys*; virginity; competition; Nicholson-Bailey; sex ratios; biological control; cabbage seedpod weevil; parasitoids;

Acknowledgements

I'd like to particularly thank my two co-supervisors, Bernie Roitberg and Dave Gillespie, for providing me with guidance, support, and inspiration throughout my time at SFU. These two taught me so much, and I will always remember grad school fondly] thanks to them. I also must strongly acknowledge Tim Haye, who was my cabbage seedpod weevil guru throughout this project. Tim provided me with lots of support and insight throughout this project and I thank him greatly for it.

This thesis would not have gone nearly as well without the hard work of three very dedicated summer students: Chandra Moffat, Jamie McKeen, and Paul Abram.

I must also thank Peter Mason and Ulli Kuhlmann, for giving me my first jobs in entomology, for encouraging me to start up this project, and for introducing me to Bernie and Dave. In addition, I must thank Jenny Cory for being on my committee and providing useful insights on the thesis. Thanks also to Vince Nealis for taking the time to act as my public examiner and putting considerable effort into some very good questions and suggestions for the thesis.

To the other members of the Roitberg lab: I think I have learned a lot since my first lab presentation. Thanks for all your help, and I hope you agree.

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Glossary

Superparasitism	Parasitism of a host larvae by more than one individual of the same species
Multiparasitism	Parasitism of a host larvae by more than one individual of at least two species
Nicholson-Bailey Model	Classic model describing populations dynamics of a parasitoid-host system with discrete generations and random parasitoid search
Stable coexistence/stability	A category of population dynamics defined by having no unique values for female parasitoid population level in the final 50 generations of the model
Chaotic coexistence/chaos	A category of population dynamics defined by having more than 16 unique values for female parasitoid population level in the final 50 generations of the model
Erratic coexistence	A category of population dynamics defined by having between 1 and 15 (inclusive) unique values for female parasitoid population level in the final 50 generations of the model
Haplodiploidy	Sex determination system of most hymentopterans whereby male offspring emerge from unfertilized eggs and female offspring from fertilized eggs.
Local Mate Competition	A theory by Hamilton (1967) describing the mechanism behind female-biased sex ratios observed in some wasp species where mating is confined to the natal patch. An adult female parasitoid exploiting a patch by herself should produce the minimum number of male offspring necessary to fertilize her female offspring, thus reducing the amount of competition for her sons and maximizing the number of daughters produced.
Classical biological control	Importation of a natural enemy species from the region of origin of a pest.

Interference	Interactions among natural enemies that reduce their host suppression efficiency
Synergistic host suppression	Cases where host suppression is improved by having multiple species of natural enemy present when compared to having the single most effective natural enemy on its own.

1: Introduction

1.1 Introduction

To a parasitoid, each host represents an opportunity to directly increase its fitness. Because finding and exploiting hosts is more closely linked to reproductive success than finding and exploiting prey, parasitoids have become a favourite model organism of behavioural ecologists studying foraging and reproductive behaviours (Godfray 1994). In an applied context, they are the most important group of organisms used in the biological control of insect pests (Mills and Wajnberg 2008). Because the level of host suppression achieved by a parasitoid is dependent on its behaviour when searching for and parasitizing hosts, sound knowledge of the parasitoid's behavioural ecology is essential for the success of any biological control program (Mills and Wajnberg 2008). In particular, an understanding of how competition influences a parasitoid's reproductive behaviour will increase both its practicality and its efficacy as a biological control agent. Competition between parasitoids that share a common host can be intense as it involves individuals of one or more species that have evolved similar strategies to exploit the same resource, in the same habitat (Boivin and Brodeur 2006). Such competition can have unpredictable effects on ecosystems, and is thus an important factor to consider when making management decisions regarding biological control. For example, competition from introduced natural enemies can disrupt control by species that are already present (Rosenheim et al. 1995). Moreover, interspecific competition between parasitoids can be highly asymmetric, with one species generally outcompeting the other (Boivin and

Brodeur 2006). This can be especially important, as the parasitoid that is the most negatively affected by competition can be most the effective at reducing host densities (Mills 1991). The presence of a particular competitor can also cause changes in foraging behaviours of the other species, such as a shift in sex ratio (Lebreton et al. 2010) or an increased patch residence time (Boivin and Brodeur 2006).

My thesis uses both an experimental and a theoretical approach to address several ecological questions that have implications for biological control. In Chapter 2, I examine competition between two parasitoids and how it may influence biological control of their shared host. The study system I used is the cabbage seedpod weevil (CSW), *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) (syn: *Ceutorhynchus assimilis*). CSW is an invasive species in North America that is native to Europe and is a major pest of canola, or oilseed rape (*Brassica napus* L. [Brassicaceae]) (Alford et al. 2003; Dosdall et al. 2006). Two species of parasitoid commonly attack CSW in Europe: *Trichomalus perfectus* (Walker) and *Mesopolobus morys* (Walker) (Hymenoptera: Pteromalidae) (Haye et al. 2010; Veromann et al. 2011). Both species are candidates for potential introduction to Canada, for classical biological control of CSW. The main questions I attempt to answer in Chapter 2 are

1. Would either a single-species or a two-species introduction be favoured for biological control of CSW?

2. How is each species affected by the presence of inter- vs. intraspecific competition?

To answer these questions I use a combination of field and laboratory experiments. Choosing between single versus multiple introduction can be a challenging

decision in classical biological control (Mills 2006). First, the risks of each option must be carefully weighed against the rewards (Bigler and Kolliker-Ott 2006). Because introducing multiple species increases the risk of negative non-target effects, in order to justify any sort of multiple species introduction a biologically significant increase in host suppression levels must be found when compared with the level of host suppression obtained by the more effective biological control agent in isolation. I thus conduct an experiment looking at how each parasitoid performs in the presence of a conspecifc vs. a heterospecific competitor. I also attempt to determine whether one species has greater host suppression ability than the other, examining parasitism rates of each species in the absence of competition. I further explore this system by looking at some of the mechanisms behind competition. Through laboratory experiments I examine the immature development of both species, both when alone on a host and when together with a heterospecific larva.

Based on my field competition experiments, I find that *T. perfectus* produces a female biased sex ratio when paired with a conspecific. I expand on this finding in Chapter 3 where I take a theoretical approach, examining the implications of this behaviour, and how this trend could affect host-parasitoid population dynamics. Additionally, I incorporate female virginity into the model. Since parasitic wasps are haplodiploid (the sex of their offspring is determined by whether or not an egg is fertilized), virgin females are restricted to producing only males. An increasingly female biased population, with all else being equal, should have increasing levels of female virginity due either to decreased encounters with males, or through other mechanisms such as male sperm depletion (Jacob and Boivin 2004). I examine how these factors

influence dynamics, as well as what implications they may have for biological control of the host.

Applied research on parasitoids of agricultural pests has produced a wealth of knowledge on the behaviour and ecology of many species (Godfray 1994). In my thesis I elucidate many aspects of the biology of the parasitoids attacking CSW, particularly the mechanisms and outcomes behind inter- and intraspecific competition. It is my hope that this thesis provides not only some useful answers to questions regarding biological control of CSW, but also stimulates further research, or at least inspiration for further inquiry, on sex ratios and virginity in parasitic wasps.

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2: Inter- and intraspecific competition between parasitoids of the cabbage seedpod weevil and their effects on behaviour and biological control.

2.1 Abstract

Two parasitoids, *Trichomalus perfectus* (Walker) and *Mesopolobus morys* (Walker) (Hymenoptera: Pteromalidae) are candidates for potential introduciton for classical biological control of the cabbage seedpod weevil in Canada. Using a combination of field and laboratory experiments, I set out examine the effects of inter- and intraspecific competition between these two parasitoids, with a focus on biological control of their common host. No synergistic effects on host suppression could be detected when both species were present. I thus suggest that a single-species introduction in Canada should be favoured over a two-species introduction. Neither species was found to be a superior competitor at the larval stage. Interestingly, I found that when paired with a conspecific female, *T. perfectus* produced a female-biased sex ratio, whereas it produced an unbiased sex ratio when paired with *M. morys*. This is the opposite of what is predicted by Local Mate Competition theory. I also describe the immature development of both species.

2.2 Introduction

The parasitic Hymenoptera are a highly successful and diverse group that has profound effects on terrestrial ecosystems (Godfray, 1994). Competition among these parasitoids for hosts can be intense; each encountered host is linked very directly to an increase in fitness for the individual parasitoid. As a consequence, many adult parasitoids are extremely effective at foraging, and can be highly specific to their hosts. Parasitoids are subject to many direct forms of competition including aggressive behaviours between conspecific and heterospecific adult females, as well as fighting and physiological suppression between larvae on a shared host (Boivin and Brodeur, 2006). Unlike prey, which are effectively removed from their habitats by predators, parasitized hosts remain in their habitats and may be encountered by new conspecific or heterospecific parasitoid females. These encounters can have results ranging from females abandoning hosts, to multiparasitism and facultative hyperparasitism. This topic is of special interest in biological control, as competition with introduced natural enemies has been shown to disrupt host suppression by endemic species, as well as affect both establishment and overall success rates in many biological control programs (Denoth et al., 2002). An in-depth knowledge of the effects of competition in a system is thus essential when making biological control decisions.

One important issue in classical biological control is whether a single or a multiple species introduction will provide a better level of host suppression. This can be very difficult to predict, as there is often a strong influence of many extrinsic factors determining the outcomes of competition (Mills, 2006). Heterospecific intra-guild interactions are often asymmetric with one species generally winning (Boivin and

Brodeur, 2006). The effects of such interactions on ecosystem dynamics can be striking. For example, the parasitoid that is most effective at reducing host densities may be the least adapted to competition, and thus the weakest competitor within a guild (Mills, 1991). Denoth et al.(2002) evaluated 108 cases of single and multiple species introductions for biological control of insect pests and found that the single species introductions were more often successful than multiple species introductions. They found higher success in establishment of the natural enemy in single species introduction, and no improvement in success of the biological control programs relative to the number of agents released. Additionally, in a majority of the successful cases where multiple species were introduced for biological control, a single species was considered to be responsible for the success.

2.2.1   Life history of the cabbage seedpod weevil and its parasitoids

The cabbage seedpod weevil (CSW), *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) (syn: *Ceutorhynchus assimilis*) is a univoltine species of European origin that is invasive in North America, and is a major pest of cruciferous crops, particularly canola or oilseed rape (*Brassica napus* L. [Brassicaceae]) (Alford et al., 2003; Dosdall et al., 2006). Adult weevils invade *B. napus* fields when flowering has begun. They cause some damage to the crops through feeding on floral buds and young seedpods, but most most of the damage to crops is through their oviposition into seedpods. CSW larvae hatch inside the seedpods and consume developing seeds. The larvae remain concealed within the seedpods until feeding is complete. They then bore a hole through the seedpod and drop to the soil to pupate (Alford et al., 2003). CSW emerge from their pupae in late summer and overwinter as adults.

CSW is commonly attacked by two parasitoid species in Europe with similar life histories: *Trichomalus perfectus* (Walker) and *Mesopolobus morys* (Walker) (Hymenoptera: Pteromalidae) (Haye et al., 2010; Veromann et al., 2011). Both species are larval ectoparasitoids, and only attack the final two instars of the CSW larvae. These parasitoids attack the concealed CSW larvae through the seedpods. They are both solitary idiobionts, immobilizing the larvae prior to oviposition. After oviposition, the parasitoid larvae will hatch and then consume the host. Pupation for both species occurs within the seedpod. The adult parasitoids emerging from the pupae will exit the seedpod by boring through the pod wall. Both parasitoid species are univoltine, and mating occurs soon after parasitoid emergence. Since males do not overwinter, there are no males present in the population during the oviposition period.

Parasitism of the CSW larvae reduces damage within the same season to some degree, but the most dramatic effects on pest control are through mortality of the larvae and subsequent reduction of CSW adults in the following year (Williams, 2003). Both *T. perfectus* and *M. morys* are candidates for potential introduction for classical biological control of the CSW in Canada.

In this chapter, I assess the effects of inter- and intraspecific competition between *T. perfectus* and *M. morys*, and attempt to determine whether one species alone or both species together would provide better biological control of the CSW. The effects of interspecific competition on biological control can be highly unpredictable, and a strong experimental examination of the effects of competition is necessary to make any decisions regarding a single vs. a multiple species introduction (Mills, 2006). In this chapter, I thus look for synergisms or antagonisms in host suppression. I hypothesize that

the two species of parasitoid together produce a differing level of host suppression than either one does alone. I also examine changes in behaviour of the parasitoids based on their competitive pairing. To further explore the effects of competition in this system, I examine whether one species is superior in its host suppression ability, or in its competitive ability. Since the reproductive biology of these parasitoids (*M. morys* in particular) is not yet very well known, I also describe the development of both species.

2.3   Methods

2.3.1   Field sites

Two winter oilseed rape (*B. napus*) fields were used as field sites, both of which were located in the Delémont valley, Switzerland (47°22'18.16"N, 7°20'20.86"E Delémont CH; 47°21'51.13"N, 7°22'22.33"E Courroux, CH). Both fields were fairly synchronized in their development, and were 2.7 km apart as the crow flies. The fields were both sprayed with insecticides against the pollen beetle, *Meligethes aeneus* (Fab.)(Coleoptera: Nitidulidae). These insecticides were applied before the blooming stage of the crop, so they are unlikely to have had any significant impact on the experiment. The experiments took place in the summer months of 2009, from early May to early August.

2.3.2   Field cage host rearing

Field cages were perforated plastic bags (27.5 x 45.0 cm) that were placed on flowering oilseed rape plants before any pods had developed. The plants were thus

protected from uncontrolled infestation by CSW present in the field, and CSW larvae were protected from parasitoids. *C. obstrictus* were obtained prior to the experiment through mass collections from nearby fields of *Brassica rapa* L. [Brassicaceae] (commercial variety: Buko), and were kept in cages in the laboratory (BugDorm-1 Insect Rearing Cage 30x30x30 cm) with oilseed rape flowers. After approximately two weeks, once pods had developed in the cages, two mating pairs of *C. obstrictus* obtained were added to each field cage. I staggered the rearing schedule, adding CSW pairs to blocks of cages approximately every two days, so that *C. obstrictus* larvae were continuously available throughout their growing season. The cages were blocked in time, based on when they were infested with CSW. Seven different blocks of field cages were used. To guide the timing of the experimental releases, I regularly sampled each block through dissection of all the pods in a randomly selected field cage within the block. I was thus able to estimate the infestation levels, and determine the stages of the CSW larvae in the pods within each block. In the first two blocks, the adult weevils were removed from the bags once a high degree of infestation had been obtained to prevent over-infestation. In the later blocks, the weevil adults were left in the field cages for the duration of the experiment, as they were laying at a slower rate and over-infestation was no longer a problem. A field cage was considered over-infested if there were frequently two or three weevils per pod, as CSW typically only lays one egg per pod (Alford et al., 2003). Once the larvae in a block were mostly in their 2nd or 3rd instars, the block was ready to use in the experiment.

2.3.3 Obtaining suitable parasitoid adults and larvae

All adult *M. morys* and *T. perfectus* females were field-collected from various sites around Switzerland. Since both are univoltine and overwinter as adults, the ages of all females were assumed to be similar, and any differences in ages of the parasitoids would have been randomly distributed among treatments. Before being used in the field experiment each female was tested to ensure it was receptive to CSW larvae and capable of laying eggs. To test this, females were individually placed in a Petri dish with a seedpod containing an unparasitized CSW larva overnight. The pod was dissected the following day to determine whether the larvae had been parasitized. The parasitized larvae from this test were used in either the reproductive biology experiment or the larval competition experiment (both detailed below). All adult females that laid eggs were kept at 10°C in containers with honey-soaked cotton until being used in the field experiments. The parasitoids that failed to oviposit on the CSW larvae were not used in the experiments.

2.3.4 Reproductive biology of *T. perfectus* and *M. morys*

Development times of both parasitoid species were determined by rearing on CSW larvae parasitized in the laboratory (as described above). After parasitism in the laboratory, any excess eggs due to self-superparasitism were removed so that only one remained on the host. In the first season (summer 2008), the parasitoid larvae on hosts were kept in small Petri dishes (2 cm diameter) with moist filter paper, following the method by Murchie &Williams (1998). This method produced a very high level of mortality due to excess moisture (91% for *M. morys* 82% for *T. perfectus*), so in the

second season the parasitized CSW larvae were kept in pipette wells on 96-well plates.

Rather than using moist filter paper, the well plates holding the parasitoid larvae in the

second season were kept in a sealed container with a saturated potassium chloride bath to

maintain approximately 85% relative humidity. In both seasons, larvae were kept at a

constant 20°C with 15 hours light and 9 hours dark. Mortality under this new method

was considerably lower (62% for *M. morys* and 46% for *T.* perfectus). Development

times from each year were pooled.

The length of the eggs was measured to determine if this was a possible way to

distinguish between the eggs of each species. The immature parasitoids were checked

daily for changes in stage until adulthood. The stages that were recorded were egg, larva,

pupa, and adult. It was not possible to conclusively distinguish between larval instars

during development, as moults were not normally visible and neither species had head

capsules.

2.3.5   Inter- vs. intraspecific competition experiment

I tested the effects of intra vs. inter specific competition on the two parasitoid

species, by infesting field cages with CSW larvae and exposing them to one of three

treatments. Treatments were two *T. perfectus* females (TT; n = 16), two *M. morys*

females (MM; n = 19), or one *T. perfectus* female and one *M. morys* female (MT; n =

20). The parasitoids were added to the field cages once each block was determined to be

in the right stage – i.e., containing mostly 2nd and 3rd instar CSW larvae. The parasitoids

were left in the cages for 3 or 4 days, depending on weather conditions, and then

removed. The fourth day was included in the exposure period when it was raining on the

third day, as in such conditions it was impossible to locate the parasitoids in the field cages due to all the moisture in and on the cage. The cages were blocked by the dates on which they had been exposed to the parasitoids. All cages within each block also shared the same CSW infestation dates, to ensure that infestation levels and host age structures were as similar as possible. Seven blocks were used in this experiment, with the wasp exposure start dates ranging from the 1st to the 18th of June.

Cages where one or both of the parasitoids had died or escaped from the field cage were excluded from the analysis. To ensure that the hosts were exposed to the parasitoids for an appropriate period of time, and that no excessive self-superparasitism was occurring, single female treatments were run in parallel (detailed below in host suppression experiment). For the single treatments, the pods were dissected after each treatment period of 3-4 days. Since self-superparasitism levels were low, I judged this to be a suitable exposure time.

Once the parasitoids were removed, I re-sealed the field cages so that no further parasitism could occur, and left them in the field for approximately 15 days so that most of the parasitoid offspring would be in the pupal stage, approaching emergence. After this period, I cut the canola plants below the plastic bag and brought them into the laboratory. I then removed all the pods and placed them in clear plastic emergence containers (d: 10cm h: 10cm). These containers were checked daily for emerging parasitoid offspring, and the sex and species of each were recorded. The number of emerging parasitoids, rather than the number of weevils killed, was used as the response variable. This was done for several reasons – an important one being that determining the exact number of weevil larvae killed in each cage would involve destructively

sampling the cage. Additionally, measuring the number of weevils killed in each

scenario would serve only as a measurement of host suppression within one generation.

The number of emerging parasitoids, however, acts as a conservative measurement of the

number of weevils killed, while also serving as an indicator of future success as a

biological control agent. I measured right hind tibia length of all parasitoid offspring for

an additional measure of fitness, as body size is a practical proxy for measuring fitness in

parasitoids (Roitberg et al., 2001). For these measurements I used an ocular micrometer

at 40x magnification. All specimens from this, and the following experiments were added

to the collection at CABI Europe: Switzerland.

2.3.5.1   Statistical analysis

Due to the concealed nature of the host, it was not possible to determine the exact

number and stage of the larvae in any given field cage without destructively sampling

them. From the dissected single parasitoid treatments (described below), I dissected all

the pods to estimate the mean number of weevils available for parasitism in each block. I

used the number of emerging parasitoid offspring as the main response variables in the

statistical tests. Larval mortality of the parasitoids was assumed to be even among the

species and was not included in the test as it was not possible to measure in this

experiment. To determine whether both parasitoid species together were more effective

as biological control agents than either one alone, I compared total wasps emerging from

each intraspecific treatment (MM, or TT) to the interspecific treatment (MT). To

examine performance of each individual parasitoid based on competitive pairing, I

compared the total emergence of each species (per cage) in the MT treatment with half of

the total emergence in the respective intraspecific treatment (MM for *M. morys*, TT for *T. perfectus*). This was done in order to compare the average number of offspring produced by a single female of either species in the presence of a conspecific with the number produced in the presence of a heterospecific. For each of these comparisons I performed a standard least squares test. The experiment was originally conceived as a randomized block design, with the dates of exposure to the parasitoids as the blocks, but since weevil density differed between blocks, the weevil density estimates were instead included as a covariate for the least squares test. Because the first two blocks had unusually high CSW densities that could have biased the results, they were excluded from the test. Inclusion of these blocks, however, did not affect the conclusions of the analysis. Any cases where one or both adults could not be found after the exposure period were excluded from the test. Additionally, any cases where no parasitoid offspring emerged were also excluded. This was done because observations indicate that zeros are problematic, rather than informative, as both species readily oviposit on hosts. Normality of residuals for each test was checked. Since the residuals from the *T. perfectus* test fit a normal distribution, the data were not transformed. For the *M. morys* test, however, the residuals did not fit a normal distribution, so the data were log transformed. A power analysis was performed for each test. Hind tibia lengths of emerging parasitoid offspring were also compared using a least squares test, with the field cage from which they emerged as a random effect. In summary, the experimental units were the field cages, blocked in time.

I compared the sex ratios produced by each species in each treatment within the inter- vs. intraspecific competition experiment using a Wilcoxon rank sum test. All instances where no parasitoids emerged were excluded from the test, since it could not be

determined whether this result was due to the reproductive inability of the adult

parasitoid or a lack of hosts – either case being sufficient reason to exclude them from the

analysis. In addition, all instances of only a single parasitoid emerging were excluded

because a sex ratio could not be estimated from a single individual.

2.3.6   Host suppression efficacy

I ran a second experiment in parallel with the competition experiment to

determine whether one species was superior to the other regarding host suppression. The

field cages were in the same blocks of weevil rearing as the experiment described above.

Treatments in this experiment consisted of single females, either *M. morys* or *T.

perfectus*, kept in the cages for 3 or 4 days in the same time blocks as above. After the

exposure period, however, the cages were cut below the bag and brought back to the

laboratory where all the exposed pods were then dissected. I recorded everything I found

in the pods including weevil eggs, healthy and parasitized CSW larvae and their instars,

the number of parasitoid eggs, and the number of parasitoid larvae. These measures were

used to estimate the mean number of CSW larvae available for parasitism in each block,

as well as to estimate the amount of self-superparasitism that might have occurred during

the experiment. These results also allowed us to determine the proportion of weevils

parasitized by females of each species when in isolation.

2.3.6.1 Statistical analysis

I used a generalized linear model with a constrained Poisson distribution to test the effects of treatment (a single *M. morys* or *T. perfectus* female) with total weevils available in each cage (eggs + larvae) added as an effect, on the total number of weevils parasitized-1. A constrained Poisson distribution was chosen because I excluded all instances where 0 weevils were parasitized, since the cause of this behaviour could not be determined (female may have been hurt, quality of plant may have been compromised, etc.).

2.3.7 Larval competition experiment

To elucidate some of the mechanisms behind competition in this system, I performed an experiment in the laboratory that examined competition at the larval level. This experiment allowed me to determine whether one species had a competitive advantage over another at the larval stage. The treatments consisted of CSW hosts parasitized with either two *T. perfectus* eggs, two *M. morys* eggs, or one from each species. I obtained the parasitized larvae and reared them in the same manner as in the reproductive biology experiment. Each host was exposed to a single parasitoid, and was examined for parasitism. If it had been parasitized, excess eggs were removed so that there was either only one remaining for the interspecific treatment, or two remaining for the intraspecific treatment. The removed excess eggs were then carefully placed on hosts that were parasitized by the other species. I kept track of which species originally parasitized each host, to determine priority effects on the outcome of competition. The parasitoid larvae were checked daily for changes in stage, and the species and sex of each

were recorded once they had emerged as adults.   To assess whether the host's having

been originally attacked by *T. perfectus* or *M. morys* had an impact on the outcome of the

larval competition, a Chi-square test was used. Once the original attacker was determined

to have no impact on the outcome, a Chi-square was also used to test whether one species

won larval battles at a greater rate than the other did. Upon pupation, the pupal lengths

were measured and compared against the pupal lengths from the reproductive biology

study (in which parasitoids were reared individually) using a least squares ANOVA to

test whether body size is affected by having two versus one immature parasitoid initially

present on the host.

2.4   Results

2.4.1   Reproductive biology of *T. perfectus* and *M. morys*

The eggs of *T. perfectus* and *M. morys* were too similar in appearance and length

to confidently be able to distinguish one species from another (Table 3.1). Both species

spent less than two days, on average, in the egg stage (*T. perfectus:* 1.41 SE 0.03 days;

M. morys 1.51 SE 0.05 days). From egg to adult emergence, *T. perfectus* females took

19.7 (SE 0.34) days, while males took 18.2 (SE 0.56) days. *M. morys* females and males

took 18.8 (SE 0.27) and 18.6 (SE 0.80) days, respectively, to emerge as adults from eggs.

Mean development times between each stage are shown in Table 3.2. My observations

regarding the development of *T. perfectus* agree with previous data showing the

development time from egg to adult lasting 19 to 25 days (Williams, 2003). The pupal

stage of *T. perfectus* in the current study was approximately 2 days shorter than the values reported by Murchie and Williams (1998); this is due to the fact that their rearing temperature was 18°C, whereas the current study had a temperature of 20°C. To my knowledge, this study is the first record of *M. morys* development times. Distinguishing one species from another was not normally possible until the pupal stage, although *M. morys* larvae differed from *T. perfectus* in that they occasionally spun a partial cocoon just prior to pupation.

2.4.2   Inter vs. Intraspecific competition field experiment

No synergistic host suppression was observed with the presence of two species; the total wasps emerging from either of the intraspecific treatments (MM or TT) were not significantly different from the interspecific treatment (MT). Details of the least squares test are summarized in Table 3.3. The average number of offspring per female that emerged from MM treatments was not significantly different from total number of *M. morys* emerging from the MT treatments (p =0.47). Likewise, the number of emerging *T. perfectus* offspring per female from the TT treatments was not significantly different from total number of *T. perfectus* offspring from the MT treatments (p=0.71) (Table 3.4). Hind tibia lengths for male or female *M. morys* or *T. perfectus* were very similar across treatments (Table 3.5), and no significant differences were found based on competitive pairing.

The sex ratio of emerging *T. perfectus* was significantly more female-biased in the TT treatment than in the MT treatment (Chi = 4.72, d.f. = 1, p =0.03). When paired with a heterospecific, *T. perfectus* produced an offspring sex ratio (proportion of females) of 0.52 (SE 0.08), whereas when paired with a conspecific, the ratio shifted to 0.72 (SE

0.05). No sex ratio shifts were detected in *M. morys* based on treatment (p=0.76). In both treatments, *M. morys* produced an offspring sex ratio that was approximately 2/3 females (MM: 0.64 se 0.05; MT: 0.66 se = 0.06).

2.4.3   Host suppression efficacy experiment

Examining the proportions of weevils parasitized by either species from the single adult treatments, I found no evidence that one parasitoid was more effective than the other was (Chi = 0.02, p = 0.88). As expected, there was a significant increase in the number of wasps produced as the numbers of weevils available increased (Chi = 37.9, p < 0.0001). This supports my use of weevil density estimates as a covariate in the competition experiment. From the laboratory-reared parasitoid offspring of the single female field treatments (with oviposition occurring in the field, and dissection of pods and rearing of offspring in the laboratory), *T. perfectus* produced a sex ratio of 0.52 SE 0.05 females, while *M. morys* had 0.56 SE 0.08 females.

2.4.4   Larval competition experiment

There was no evidence for a priority effect on the outcome of the larval competition (Chi = 0.24, p = 0.63). This also indicates that my transferring of parasitoid eggs to artificially multiparasitize the hosts did not damage the eggs. *M. morys* and *T. perfectus* did equally well in the larval stage when matched up against one another, with each species winning 50% of the interspecific larval battles (n=46, p = 1). I did not find any significant sex ratio shifts in the larval competition experiment based on competitive

pairing (conspecific versus heterospecific) for either species. There appeared to be a slight female bias in secondary sex ratio of *T. perfectus* when competing with either *T. perfectus* or *M. morys* (TT and MT treatments, pooled) compared to when alone, but the difference was not significant (p = 0.07). Pupal sizes in the competitive treatments were not significantly different when compared with those developing in the lab in the absence of competition (*T. perfectus* males: p = 0.70, females: p =0.40; *M. morys* males: p=0.46, females: p = 0.20). Offspring of both species were highly mobile when newly emerged from the egg. The first instars would move about the host, killing other eggs or other larvae that they encountered. After this mobile period, the parasitoid larva began consuming the host.

2.5   Discussion

This study examined many aspects of cabbage seedpod weevil (*C. obstrictus*) parasitoid biology that are highly important for decisions regarding the biological control of this pest. Beyond addressing the primary questions concerning inter- versus intraspecific competition, my observations of the development times of both *M. morys* and *T. perfectus* will facilitate further work with these species. Furthermore, the interesting finding of a female-biased sex ratio shift by *T. perfectus* in response to competition opens the door to future theoretical and experimental inquiry.

2.5.1   Development of *T. perfectus* and *M. morys*

Both species had similar developmental trajectories, including a short egg stage and an aggressive first instar. Chemical cues from fresh frass of CSW larvae have been

shown to be a cue that elicits oviposition in *T. perfectus* (Dmoch and Rutkowskaostrowska, 1978), and there is some preliminary evidence that fresh frass may also be a cue for *M. morys* (Kuhlmann et al., 2008). Dry frass, however, does not appear to elicit oviposition for either species (Kuhlmann et al., 2008). This suggests that once a host has been parasitized and is no longer defecating, there is only a short window of time during which an additional parasitoid could detect and multi- or superparasitize the host. A parasitoid would thus be much more likely to experience competition during its egg and first instar stages than during later larval stages. This may explain both the short egg stage, as well as the aggressive first instar of both species. If the fresh frass is indeed the only cue used by ovipositing females to detect hosts, later parasitoid instars would not be subject to competition and aggression from other larvae, and thus would derive no fitness advantage from being aggressive themselves.

2.5.2   Single vs. multiple introduction in Canada

From these experiments, it appears that multiple species introduction for classical biological control of the cabbage seedpod weevil (CSW) would not provide additional benefits over a single species introduction. Host suppression was not improved by having two species of parasitoid present when compared to having just one. In order to justify the extra risk, as well as additional cost associated with introducing two species, I would have had to have observed a strong synergistic response in host suppression with both species present.

We must nevertheless be careful in interpreting these negative results, as the power of the intra- vs. interspecific test was fairly low (*T. perfectus* test: power = 0.115; *M. morys* test: power =0.112). I therefore cannot conclude that there are no synergistic or

antagonistic effects on host suppression, as I may have failed to detect them (Hoffmeister et al., 2006). Nevertheless, the fact that any synergisms that may be present were not strong enough for us to detect supports my conclusion that a single-species introduction should be favoured over a two-species introduction. As these experiments were small-scale, cage experiments, they likely ignore some population-level processes, such as the effect of the two species invading the field at different times. Further experiments comparing parasitism levels in fields where only one species is present to those where both species are present could help address this question.

From another standpoint, I cannot rule out any antagonistic interactions that could occur with species already present in Canada. Gillespie et al. (2006) found parasitism rates on CSW by species already present in Canada to be relatively low at 2-4%. Any antagonistic effects caused by introducing *T. perfectus* or *M. morys* would thus be unlikely to reduce host suppression much beneath its current levels. Moreover, the two most common species attacking CSW in Canada were *Trichomalus lucidus* and *Stenomalina gracilis,* which are both present in Europe (Gillespie et al., 2006; Haye et al., 2010; Ulber, 2003; Williams, 2003).

Another factor that should be taken into account is the potential influence of the field cages themselves. The main issue with the field cages is that they restricted the parasitoids to one patch. This may be significant as patch residence time in parasitoids can be highly plastic (Roitberg et al., 1992). A parasitoid experiencing competition from another species could, for example, decrease its patch residence time or even abandon the patch altogether. Further research on patch exploitation behaviour in these species in the presence, and absence of competition could prove interesting.

2.5.3 Ideal candidate

Despite the obvious importance of adequate host-specificity, many other criteria must be taken into consideration before introducing a species into a new area as a classical biological control agent. One essential trait is that such an agent must be able to reduce host densities to a biologically significant degree. This important benefit must be balanced with the environmental risks of introducing the agent (Bigler et al., 2006). All else being equal, a species that is superior in suppressing its host will provide more benefits, and thus is a better candidate for introduction. I did not, however, find one species to be any more efficient than the other at host suppression. Although this may have been due to insufficient power in my tests, both parasitoid species are found to parasitize *C. obstrictus* at similar rates in Switzerland (Haye et al., 2010). *Trichomalus perfectus,* however, has been found to be more abundant than *M. morys* in other regions of Europe (Williams, 2003), such as Estonia (Veromann et al., 2011). This indicates that *T. perfectus* may be superior at dispersing to and surviving in a wider range of environments, and thus may have better establishment success if introduced into Canada. Molecular work has found that the CSW's two introductions to Canada originated from 1) either western or northern Europe, and 2) either Sweden or Russia (Laffin et al., 2005), which further supports *T. perfectus* as a candidate, as it is the most widespread parasitoid covering those regions of origin.

Of particular consequence is the fact that *T. perfectus* has been recently discovered in Quebec (Mason et al., In Press). Although this does not remove the importance of appropriate testing before intentionally introducing *T. perfectus* into other

regions of Canada, it strongly supports a concentration on *T. perfectus* as the main

candidate, rather than *M. morys*, as the marginal risk of introducing *T. perfectus* has been

reduced. Since *T. perfectus* is already present, much of the potential non-target impact of

this parasitoid in North America is now out of our control. The options for biological

control of CSW, other than introducing neither species, are now to either intentionally

introduce *T. perfectus* into other areas of Canada (that it may eventually spread to even

without introduction), or to introduce *M. morys,* and take on the risks associated with

having both species present. Therefore, unless establishment and control of CSW by *T.*

perfectus is found to be a failure in Canada, it will be difficult to justify an introduction of

M. morys, as it does not appear to demonstrate superior host suppression ability.

2.5.4   Parasitoid fitness

Emergence of adult parasitoids was similar under both heterospecific and

conspecific competition for both species. There was also no competitive advantage at the

larval level to being one species or the other. For both species, multi- or superparasitism

likely does not decrease the amount of food available to the winning parasitoid offspring

as competing parasitoid larvae and eggs are rapidly killed so that only one remains. In

fact, self-superparasitism, or laying multiple eggs can be a viable strategy for a female

deciding to attack a previously parasitized larva, since it can increase the odds of one of

her offspring winning the host (Boivin and Brodeur, 2006; Godfray, 1994) as well as

provide additional food for her potentially winning offspring.

2.5.5 Sex ratios

Perhaps the most interesting finding of this study was the female-biased sex ratio observed in *T. perfectus* in response to a conspecific competitor. The sex ratio of the emerging *T. perfectus* offspring was significantly more female biased in the intraspecific competitive scenario than it was in the interspecific scenario (72% females versus 52% females, respectively). As I was only able to determine the final sex ratio of the emerging offspring, explanations behind the mechanism of this sex ratio shift remain speculative. The observed sex ratios could be due to maternal behaviour, differential mortality of male and female larvae, or a combination of both these factors. Although sex ratio shifts can be caused by a number of factors such as host size, the sex ratio shifts I observed were in response to the manipulations, as any other sex ratio distortions would be randomly distributed between treatments in the experiment.

Parasitic Hymenoptera have been used extensively in sex ratio studies, due in part to their haplodiploid sex determination. Male offspring develop from unfertilized eggs and females from fertilized ones, providing a mechanism by which adult females are able to control the sex of their offspring in order to maximize their fitness (Godfray, 1994). The sex ratio shift I observed in *T. perfectus* is particularly interesting because it is in the opposite direction of sex ratio shifts that are usually predicted in haplodiploid parasitoids. Local mate competition (LMC) is the most common explanation for female-biased sex ratios in parasitic Hymenoptera. LMC (Hamilton, 1967) occurs when mating is confined to the natal patch, and thus any male offspring in a patch, including brothers, will be competing against one another for mates. When an adult female parasitoid exploits a patch by herself, she should lay the minimum number of male offspring necessary to fertilize her female offspring, thus reducing the amount of competition for her sons and

maximizing the number of daughters produced. The ideal sex ratio shifts back towards

the 50:50 sex ratio (as originally explained by (Fisher, 1958)) when mating is no longer

confined to the natal patch, or when the number of foundresses increases. The sex ratio

shift I observed in my experiments is therefore not explained by LMC, as there was a

shift towards a more female-biased sex ratio when a conspecific adult female was

present, rather than absent.

Another common mechanism affecting haplodiploid parasitoids sex ratios is

referred to as environmental sex determination, where the relative fitness of one sex is

different in certain situations. Host size, or host quality can often explain a sex ratio bias,

wherein males tend to lose less relative fitness when developing on a lower quality, or

smaller host (Charnov et al., 1981; King, 1987, 1993). This is a likely mechanism behind

why I observed a consistently female-biased sex ratio in *M. morys* across treatments, as

the CSW hosts were only exposed to the parasitoids once they were fairly well developed

in their third or late second instars, and thus could be considered high-quality hosts.

Although the relative sizes of larval hosts has been shown to influence sex ratio of *T.

perfectus* (Murchie and Williams, 1998), this particular mechanism is unlikely to be the

driving force behind the observed sex ratio shift I observed. While the presence of a

competitor on a host could reduce its quality, I did not observe any changes in body size

of emerged parasitoids based on competitive matchup in the field experiment. I also did

not observe a difference, based on the absence or presence of competition, in pupal sizes

of developing parasitoids in the lab. As discussed above, whether or not a host has been

previously parasitized likely does not affect its food resource value because early instars

do not begin consuming the host until after killing any competitors present.

If the female larvae of *T. perfectus* are stronger competitors than the male larvae, this difference could potentially explain a female bias in either the primary or secondary sex ratio of this species. Differential competitive abilities of male and female larvae have been documented in several species (Lebreton et al., 2010; Van Baaren et al., 1999). In some species, one sex may have enhanced physical features such as larger mandibles, or enhanced setae to improve mobility (Van Baaren et al., 1999). In this study, there were no obvious visible differences between male and female larvae, although these observations do not exclude gender-based differences in competitive behaviour. Lebreton et al. (2010) found a mechanism for a similar female-biased sex ratio shift in the parasitoid *Anisopteromalus calandrae*: male larvae were inferior competitors, and ovipositing females thus laid mostly female eggs when superparasitizing, given that female offspring were more likely to survive. The decision of a superparasitizing female may be further influenced by the sex of the offspring on the previously parasitized host. A host previously parasitized with a female offspring represents a potential mating opportunity for one of her sons. If she lays a male egg and it wins the larval competition, its fitness will be slightly reduced since it has removed a female that it could have potentially mated with (Lebreton et al., 2010). A female parasitoid may thus be more likely to lay a female egg in this scenario. Although death from losing a larval battle clearly has a greater impact on fitness than a lost potential mating opportunity, (Pickering, 1980) pointed out that when dealing with related individuals, males receive a greater benefit vs. cost in behaving altruistically towards a sister than she will due to asymmetries in relatedness. Female larvae may thus be more inclined to act aggressively towards males, which could contribute toward asymmetries in competitiveness. Because

the field experiment did not allow the ovipositing female to leave from the plant following oviposition, the chances of self-superparasitism were increased, which may have increased the likelihood of altruistic behaviour by males as a consequence. However, self-superparasitism from the non-competitive treatments was fairly low for *T. perfectus* (9.2% of parasitized weevils had more than one egg), so competing larvae in the TT treatment were probably unrelated most of the time.

Whatever the mechanism, the trend is an intesting one. The sex ratios of *T. perfectus* are highly plastic, which could have implications for biological control. For example, female-biased offspring due to crowding effects upon initial releases could improve establishment success. On the other hand, increased competition for hosts in one season should lead to an increase in the proportion of females produced, which could further increase competition for hosts in the following season. This may result in a potentially unstable positive feedback loop that could produce interesting dynamics. I thus decided to add this behaviour into a population model to examine its effects on dynamics and biological control. This will be explored in the following chapter.

I have shown that a strong female bias can occur on a local scale in response to increased competition. It is still uncertain, however, whether this behaviour will scale up to the population level. Murchie and Williams (1998) found that sex ratios varied significantly between the three fields they examined in 1993 and 1994. They postulated that this variance was caused by differences in the host age and size difference between fields, as female offspring tended to emerge from larger hosts. From my experiments, however, it seems possible that the variance in sex ratios could have been caused by varying levels of competition between fields. Further study at the field-scale would

provide useful insights. For example, a comparison of parasitoid and host population

sizes in the field, followed by a survey of the population sex ratios over several seasons,

could provide interesting results if sex ratios varied considerably from field to field.

2.6 Tables

Table 2.1   Mean egg sizes for *M. morys* and *T. perfectus* from a laboratory development study. Egg sizes were too similar to confidently establish species identity from egg measurement alone.

Parasitoid species	N	Mean egg size (mm)	Std error
Mesopolobus morys	118	0.369	0.026
Trichomalus perfectus	260	0.394	0.024

Table 2.2   Development times in days for *T. perfectus* and *M. morys* for each immature stage from initial parasitism to emergence. Throughout their development, the parasitoids were kept at 20°C and 15 hours of light per day.

	Egg to hatching	Hatching to pupation		Pupation to emergence		Total development (egg to adult)	
	(♀+♂)	♀	♂	♀	♂	♀	♂
T. perfectus	1.41 (se 0.03; n = 314)	8.6 (se 0.14; n = 94)	8.1 (se 0.19; n = 47)	9.9 (se 0.08; n = 129)	8.8 (se 0.10; n = 95)	20.0 (se 0.20; n = 75)	18.0 (se 0.37; n = 29)
M. morys	1.59 (se 0.05; n = 152)	8.9 (se 0.2; n = 42)	9.3 (se 0.34; n = 18)	9.0 (se 0.07; n = 78)	7.7 (se 0.11; n = 41)	19.1 (se 0.27; n = 27)	18.8 (se 0.61; n = 12)

Table 2.3   Comparisons for *M. morys* of total wasps emerged between treatments in the inter- vs. intraspecific competition field experiment. Having *T. perfectus* present with *M. morys* did not increase overall host suppression when compared to having *M. morys* alone.

Total wasp emergence test for *M. morys* (MM vs. MT)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	0.88	0.29	0.45
				Prob > F
Error	34	22.18	0.65	
C. Total	37	23.06		0.72

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.55	0.60	4.21	0.0002
Treatment[MM]	0.098	0.13	0.75	0.46
Weevil density estimates	-0.032	0.044	-0.73	0.47

Effect Tests

Source	DF	Power	F Ratio	Prob > F
Treatment	1	0.11	0.56	0.46
Weevil density estimates	1	0.11	0.53	0.47

Table 2.4 Comparisons for *T. perfectus* of total wasps emerged between treatments in the inter- vs. intraspecific competition field experiment. Having two species present did not increase overall host suppression when compared to having *T. perfectus* alone.

Total wasp emergence test for *T. perfectus* (TT vs. MT)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	3.83	1.28	1.50
				Prob > F
Error	32	27.21	0.85	
C. Total	35	31.04		0.23

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.61	0.74	3.55	0.0012
Treatment[MT]	0.12	0.15	0.76	0.45
Weevil density estimates	-0.062	0.054	-1.14	0.26

Effect Tests

Source	DF	Power	F Ratio	Prob > F
Treatment	1	0.11	0.58	0.45
Weevil density estimates	1	0.20	1.29	0.26

Table 2.5   Field experiment comparing intra- vs. interspecific competition. Results from the least squares test comparing the number of offspring per adult female emerging from the TT and MM intraspecific treatments (i.e. half the total offspring emerging from those treatments) with the total offspring of their respective species emerging from the interspecific treatments (MT). No effect of treatment was found, indicating that these species do not perform differently when paired with a conspecific than with a heterospecific competitor.

Test for *T. perfectus*, comparing ½ TT vs. MT
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	125.79	41.93	2.08
Error	28	565.08	20.18	**Prob > F**
C. Total	31	690.88		0.13

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	11.96	3.83	3.12	0.0041
Treatment[MT]	-0.30	0.80	-0.37	0.71
weevil density estimates	-0.55	0.28	-1.96	0.061

Effect Tests

Effect	DF	Power	F Ratio	Prob > F
Treatment	1	0.065	0.14	0.71
Weevil density estimates	1	0.47	3.82	0.061

Test for *M. morys*, comparing Log(½ MM vs. MT)
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	1.82	0.61	0.80
Error	32	24.40	0.76	**Prob > F**
C. Total	35	26.22		0.50

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.20	0.66	3.34	0.0021
Treatment[MT]	0.11	0.15	0.72	0.48
weevil density estimates	-0.063	0.048	-1.31	

Effect Tests

Source	DF	Power	F Ratio	Prob > F
Treatment	1	0.11	0.52	0.48
Weevil Density estimates	1	0.24	1.71	0.20

Table 2.6   Mean tibia lengths of offspring produced by *T. perfectus* and *M. morys* for each competitive treatment in the field study examining the effects of inter- vs. intraspecific competition on the two species (TT = two *T. perfectus* females; MT = one *T. perfectus* and one *M. morys* female; MM = two *M. morys females*). Tibia lengths observed across treatments were not significantly different, indicating that fitness of emerging offspring was not negatively affected by the presence of the other species.

	Tibia length in mm (se)		
Treatment	TT	MT	Treatment Effect F-Ratio (p-value)
T. perfectus males	0.506 (0.006)	0.498 (0.007)	0.59 (0.45)
T. perfectus females	0.666(0.005)	0.670 (0.006)	0.60 (0.45)

	Tibia length in mm (se)		
Treatment	MT	MM (intraspecific)	Treatment Effect F-Ratio (p-value)
M. morys males	0.487 (0.006)	0.485 (0.006)	0.50 (0.48)
M. morys females	0.599 (0.005)	0.588 (0.005)	0.71 (0.40)

Table 2.7 Grand totals, pooled by treatment, of male and female offspring of *M. morys* and *T. perfectus* emerging from the inter- vs. intraspecific competition experiment. *T. perfectus* produced a significantly more female biased sex ratio when paired with another conspecific competitor (TT treatment) than when paired with *M. morys* (MT treatment) (Chi = 4.72, d.f. = 1, p =0.03). The sex ratio produced by *M. morys*, however, was consistent across treatments.

Treatment	TT	MT	MM
T. perfectus males	74	54	-
T. perfectus females	121	55	-
M. morys males	-	47	103
M. morys females	-	87	188

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3: Female-biased sex ratio shifts in a solitary parasitoid and their effects on virginity, population dynamics, and biological control

3.1 Abstract

Mated female parasitic wasps can control their sex ratios by controlling which eggs are exposed to sperm. Virgin females, however, are restricted to producing only male offspring, as all their eggs are unfertilized. Working from a Nicholson-Bailey base model, I incorporate the effects of plastic parasitoid sex ratios. I show that an increasing female bias in response to competition can produce increased chaos and have varying effects on host suppression. I also incorporate the effects of virginity due to a lack of males. This can have stabilizing effects on parasitoid-host population dynamics, as well as prevent runaway female bias in a parasitoid population. I find that the changes in host suppression caused by these new behaviours can be either positive or negative, and are highly dependent on the search efficiency of the parasitoid.

3.2 Introduction

The evolutionary fitness of parasitoids is bound in a very direct way to situation-dependent behaviour. Because of this tight link between fitness and the decisions parasitoids make, these organisms have become popular systems of study among behavioural ecologists (Godfray 1994). One aspect of parasitoid behaviour that has been particularly well-studied is sex ratio allocation. Since most parasitoids are Hymenoptera and reproduce via arrhentokous parthenogenesis, unfertilized eggs develop into haploid

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males and fertilized eggs develop into diploid females (Cook 1993; Godfray 1994). Many parasitic wasps can control which eggs are fertilized and thus choose the sex of each offspring (King 1993), which makes hymenopteran parasitoids ideal for sex ratio studies.

Since Fisher explained equal sex ratio investment in the majority of species (Fisher 1958), sex ratios have been a well-studied area of biology. For example, in an exception-that- proves-the-rule paper, Hamilton (1967) explained why there can be strong female biases, especially in gregarious parasitoids, due to what is now called Local Mate Competition (LMC) theory. LMC predicts that an ovipositing female should produce the minimum number of males required to fertilize her female offspring. This theory assumes mating is confined to the natal patch, thus offspring dispersal prior to mating, or an increasing foundress number will shift the optimal sex ratio back toward Fisher's 50:50 ratio. Other factors that can affect sex ratio in parasitoids include host quality (Charnov et al. 1981), and differential mortality of male and female offspring due to inequalities in competitive ability (King 1993) or for other reasons such as infection with the male-killing *Wolbachia sp.* pathogen (Werren 1997). Cases like these where there are differences in fitness between the sexes in certain scenarios, combined with LMC are certainly the two most important factors affecting sex ratios of parasitoid wasps. Oviposition by virgin females, however, whose offspring are all haploid males, is another factor that could affect population sex ratios (Godfray and Hardy 1993).

Although there are some exceptions (Fauvergue et al. 1998; King 2002; Fauvergue et al. 2008; Metzger et al. 2008), the effects of virginity, especially on populations of solitary parasitoids, have not been considered in great depth. Much of this lack of depth may be due to the difficulties in assessing levels of virgin oviposition in the

field. This can be most easily assessed in species of Hymenoptera whose mating is constrained to the natal patch, such and fig wasps (West et al. 1998) and many gregarious parasitoids (Kapranas et al. 2011). When attempting to assess virgin oviposition of solitary parasitoids in the field, however, simply surveying the number of virgins in a population is not sufficient. Since virgin females could be seeking mating opportunities, rather than seeking to oviposit, the number of virgins in a population at any given time does not necessarily represent an accurate measurement of virgin oviposition (Godfray and Hardy 1993; Fauvergue et al. 2008). These virgin females thus face a trade-off scenario where they must decide to either lay exclusively male offspring and gain fitness immediately, or search for a mate to gain the ability to produce daughters later on. In addition, mate-calling and host-searching behaviours may not be mutually exclusive activities (Fauvergue et al. 2008). In some systems, however, such as the parasitoids attacking *C. obstrictus* described in the previous chapter, there is no temporal overlap between mating and host-searching. In these systems, virgin females will always oviposit all-male offspring, as no further mating opportunities can arise (Godfray 1990; Godfray 1994).

The level of virginity in a population is affected by how effectively males locate females, which in turn is affected by many factors including weather and population density (Godfray and Hardy 1993). Virginity levels are thus likely to vary between generations. Unless mated females compensate for virgin oviposition by producing proportionately more daughters, an increased proportion of virgins in a population will increase the proportion of males in the following generation. The level of virginity in a population could also be affected by the current population sex-ratio. If a population

becomes increasingly female-biased, levels of female virginity should increase due to decreased encounter rates with males. Male parasitoids can also become sperm-depleted, which can result in unfertilized females (Jacob and Boivin 2004). A female-biased parasitoid population could be more efficient from a biocontrol perspective, but in addition to potentially increased levels of female virginity, this higher parasitoid efficiency might reduce stability in the system (Beddington et al. 1978; Mills and Getz 1996).

In this chapter I examine some of the consequences that a solitary parasitoid species might experience when producing a female-biased sex ratio. I examine effects on parasitoid-host population dynamics, as well as on biological control of the host population. I propose that virginity can act as a stabilizing mechanism on parasitoid-host dynamics and prevent runaway female-bias in a parasitoid population.

3.3 Model

I modified a well known parasitoid-host model so that any unusual dynamics could be explained by the addition of the sex ratio shift. The Nicholson-Bailey model is appropriate for modelling a system like that of *C. obstrictus* as both the host and parasitoid are synchronized in discrete generations (Mills and Getz 1996). I chose to build upon the classic Nicholson-Bailey (NB) model with the logisitic host growth term proposed by Beddington, et al. (1978), as it is a more realistic and stable system than the original NB model since hosts self-limit their growth. Comins and Welling (1985) also used this as a base model for examining sex-ratio related dynamics since it is already

stable under a range of parameters. Thus, destabilizing effects can be attributed to changes added to the base model.

$$H_{t+1} = H_t e^{r\left(1 - \frac{H_t}{K}\right)} - aP_t$$

H_t represents the host population at time t. The host's intrinsic reproductive rate in the absence of competition would be equal to R. Since we are working with the logistic host growth, $r = \ln(R)$, and K is the carrying capacity for the hosts. P_t is the female parasitoid population at time t, and a is the search efficiency of the parasitoid, or the proportion of the environment that a single parasitoid can search in a generation.

The host equation remains unchanged from the Beddington, et al. (1978) model. I altered the female parasitoid equation by introducing both sex ratio shifts, and the effects of virginity:

$$P_{t+1} = \left[\sigma + (1 - \sigma)\chi^{\gamma}\right] H_t \left(1 - e^{-aP_t}\right) \theta$$

where σ is the baseline sex ratio in the absence of competition. This term was included so that sex ratio could be easily varied while keeping the range of possible proportions of one sex between 0 and 1. The shape of the curve that determines the sex ratio produced is modified by the sensitivity to competition term, ɣ. Small values of ɣ indicate a high sensitivity to competition, producing a strong female bias as competition for hosts increases, whereas high values of ɣ indicate insensitivity to competition, and very little sex ratio biasing away from the baseline sex ratio, σ (Figure 3.1). χ is the measurement of competition intensity and it is equal to the proportion of superparasitized hosts i.e. hosts containing more than one egg, and yielding an offspring of a mated female. I chose

this as the measure of competition because it relates strongly to the search efficiency of the parasitoid and is often tracked by female parasitoids in nature (Visser et al. 1992). This approach ignores the intensity of superparasitism, such that hosts parasitized by 2 or more eggs are all treated the same. χ is defined as:

$$\chi = \left[1 - \left(aP_t e^{-aP_t} + e^{-aP_t}\right)\right]\theta$$

The $aPte^{-aPt}$ and e^{-aPt} terms represent the p_1 and p_0 terms, respectively, from a Poisson distribution, and thus summed, represent the proportion of hosts not superparasitized (i.e. 'parasitized' by one or zero eggs). By subtracting this sum from 1 we get the total proportion of hosts parasitized by 2 or more eggs.

Since only mated females can alter the sex of their offspring, I defined χ to represent only those superparasitized hosts yielding the offspring of a mated female. To do this I included θ in the equation, which represents the proportion of adult females that are mated.

$$\theta = 1 - e^{-\left(\frac{M_t}{P_t}\right)\lambda}$$

The proportion of females that are mated depends on the ratio of males to females, as well as λ, which can be thought of as male mating efficacy. The equation produces positive, monotonic curves such as those in Figure 3.2, where high values of λ result in low levels of virgin females, even when males are scarce, and low values of λ result in greater proportion of virgin females in the population.

My model differs greatly from most NB-based models because it includes the male parasitoids:

$$M_{t+1} = \left[1 - \sigma + (1 - \sigma)\chi^\gamma\right]H_t\left(1 - e^{-aP_t}\right)\theta + H_t\left(1 - e^{-aP_t}\right)(1 - \theta)$$

The male equation can be broken down simply, as the first term represents the males produced by mated females, while the second term is the total offspring produced by unmated females.

3.3.1   Model Assumptions

This model assumes no competitive advantages to being male or female at the larval stage. All sex ratio shifts in the model are the result of maternal decisions. Apart from the sex ratio itself, offspring from mated and unmated females are treated the same. Like the classic NB model, mine also assumes random searching by the parasitoid, and a random, independent oviposition of eggs. This allows us to make the assumption that the emerging offspring on superparasitized hosts are distributed between mated and unmated females based on their proportion in the population (θ), and thus θ can be included in the equation for χ. The model is a well-mixed system, without any effects of local mate competition or patchily distributed hosts or parasitoids. Mated and unmated females have equal foraging efficiencies and are only different in their ability or inability to produce female offspring.

3.3.2   Iterations

For each set of parameters, the model was run for each combination of values for sensitivity to competition (γ) and male mating efficacy (λ). Values of γ varied from 0.01 to 1.1 (Figure 3.1) and λ varied from 0.1 to 8 (Figure 3.2). Some high values for both γ and λ were explored in addition. Each time, the model was run for 200 generations. The model was also run for several values of parasitoid search efficiency (*a*) and host reproductive rate (*R*).

3.3.3 Classification of dynamics

Dynamics were evaluated by counting the unique, non-repeating values for

parasitoid number (P_t) in the final 50 generations of the model. Each of the final 50

values of P_t were rounded to the nearest integer and compared to the other 49 values. If

that rounded value occurred only once, it was considered unique. That iteration of the

model was then classified into one of 5 categories: both host and parasitoid extinct (H_t

$=0$, $P_t =0$), parasitoid only extinct ($H_t \neq 0$, $P_t =0$), stable dynamics (no unique values),

erratic coexistence (1 to 16 unique values), or chaotic coexistence (more than 16 unique

values). Working with another NB-based model, Singh et al. (2009) defined a parasitoid-

host equilibrium as stable only if the host equilibrium density increased as its

reproductive rate increased. I instead chose to define stable coexistence as having no

unique values in the final 50 generations since it fit within the classification structure for

the other dynamics described above. Moreover, finding stability was not of primary

interest, as the Beddington, et al. (1978) is inherently stable across many parameters. This

definition of stability is not synonymous with stable equilibrium, because population

cycles with repeating values were considered stable.

3.3.4 Measurement of host suppression

I measured host suppression by comparing host densities in the new model to

those in the classic model. I looked at the mean host population in the final 50

generations of the classic model, and compared it to the mean host population in the final

50 generations of the new model. Another approach used by many other authors

(reviewed by Mills and Getz (1996)) is to use equilibrium densities. This approach is

equivalent to using the mean if the model indeed reaches a stable equilibrium. Quite often, however, the modifications to the NB model produced dynamics where equilibrium was not reached. Since these dynamics were of interest, I chose to use mean densities rather than the equilibrium densities to examine host suppression.

3.4 Results

3.4.1 Classic effects on dynamics

Changes in search efficiency of the parasitoid, a, had strong effects on the dynamics of the system. Similar to the classic NB model, and in keeping with recursion-based predator-prey models, an increasing a-value increased the size of the chaotic regions in the plots, as well as the regions where the parasitoid went to extinction. Increasing the reproductive rate of the host while holding a constant tended to increase the regions of chaos as well, as predicted by the Paradox of Enrichment (Rosenzweig 1971), although in some cases it reduced areas of extinction in the graph by preventing the overexploitation of hosts by highly efficient parasitoids (eg. Figs.3.3, iii and vi)

3.4.2 Novel effects on dynamics

When both sensitivity to competition (ɣ) and male mating efficacy (λ) values were very high, i.e. when females were very insensitive to competition and males were highly effective at mating with females, the model was, for all intents and purposes, identical to the classic model. This provides a good internal control, showing that the new model structure, in and of itself, did not cause novel results. Consider first when there is no virginity (with high values of λ). As sensitivity to competition increased

(moving from high ɣ to low ɣ), the dynamics tended to move from stability towards chaos. Figure 3.5 illustrates an example where there is no virginity due to very high values of λ; reading from right of the figure towards the left, there is an area of stability, followed by erratic coexistence, followed by increasing chaos. In some examples, such as Figure 3.3.vi, there is also a region of parasitoid extinction that occurs with extreme values of sensitivity to competition. In terms of dynamics, increasing sensitivity to competition produces effects similar to increasing the search efficiency (*a*) of the parasitoid in the base, Beddington, et al. (1978) model. The sex ratio shift also parallels an increased conversion efficiency (*c*) since more daughters are produced per host.

In most cases, virginity tended to lead to broader regions of stable dynamics. The only exception was with very low male mating efficacies, which produced extinction of the parasitoid. Regions on the left of Figure 3.4 with extreme sensitivity to competition (ɣ = 0.01), even with very high male efficacy (λ > 7), there was still a narrow region of stability. This stable region represents instances where the female parasitoid population as a whole has lost control of the offspring sex ratio due to substantial virgin oviposition. With decreasing λ, and thus increasing virginity, the dynamics became stable for more values of ɣ, and there was a narrowing of the chaotic regions.

Figure 3.3 demonstrates the behaviour of the model with a few different values of host reproductive rate (*R*) and parasitoid search efficiency (*a*). In the absence of parasitoids, the hosts self-regulate their growth due to the carrying capacity added to the classic Nicholson-Bailey model by Beddington et al. (1978). Figure 3.3.i illustrates a case where parasitoids have a low search efficiency. Most regions exhibit stable dynamics with the exception of the region at the bottom of the graph where the parasitoid goes

extinct. This extinction is due to the low male mating efficacies, which when combined

with the low search efficiency results in too few females being produced for the

parasitoid population to survive. The grey, extinct area gives way to stable dynamics as

the female-biasing behaviour becomes stronger and the parasitoids become more efficient

as a consequence. This extinction area also disappears when host reproductive rate is

increased, as shown in Figure 3.3.iv. In this case, although the parasitoids are fairly

inefficient, the self-limiting host is abundant enough that even with low male mating

efficacies and inefficient parasitoids, enough females are produced to maintain a stable

parasitoid population.

In most examples, there was a distinct arcing pattern to the behaviour regions, as

in Figure 3.4. The pattern is also visible in the three-dimensional host suppression plots

(Figure 3.6). Each arch indicates a region in which the populations are behaving in a

similar way. Since the sex ratio shift was counterbalanced by the degree of virginity, as

sensitivity to competition increases ($\gamma \rightarrow 0$), higher levels of virginity are necessary to

balance out the increased proportion of daughters produced by mated females. Reading

from the right of the graph to the left, at first, for an increase in sensitivity to competition

(i.e., a decrease in γ), a decrease in male mating efficacy (λ) is needed to balance it out.

However, in the middle of the green and red region of the graph, the arc reaches a vertex.

This vertex indicates the point where the decrease in male mating efficacy necessary to

balance the increase in sensitivity to competition is equal to zero. At this point, the sex

ratio biasing towards more daughters is having enough of an effect on virginity levels in

the population that in order to maintain similar dynamics, an increase in male mating

efficacy is necessary to make up for further increases in sensitivity to competition.

The arcing pattern was perhaps most distinct in the stable veins in Figure 3.4. This region, despite having fairly strong sex ratio shifts remains stable. This is interesting considering it is surrounded by increasingly chaotic regions on all sides. The populations in this arc were oscillating at fairly large amplitudes, but they were considered stable since there were no unique values in the final 50 generations. These large oscillations, although in balance in the model, are largely an artefact of the way I interpreted stability. This region would likely move into chaotic dynamics in a natural situation with increased variability in the system and is thus similar to the unstable equilibrium that we see in the original NB models, where deviation from the equilibrium point will cause the populations to oscillate at increasing amplitudes.

3.4.3 Host suppression

Host populations in the model were controlled either by the parasitoids or by a reduced reproductive rate determined by the carrying capacity (K). The ability of the parasitoid to alter its offspring sex ratio produced changes in overall host suppression, and these changes disappeared as values of ɣ and λ increased (i.e. when females were insensitive to competition and most females were mated). In such cases, the sensitivity to competition and virginity levels were low enough that the model was essentially equivalent to the classic Beddington et al. (1978) model.

Host suppression levels varied considerably based on the search efficiency (a) of the parasitoid. For example, in Figure 3.7, the only difference in parameters is the search efficiency of the parasitoid. Where $a = 0.02$, there was a general increase in host suppression as sensitivity to competition and mating success increased. In the second example, however, where $a = 0.025$, this trend disappeared, and host populations were

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mostly higher than those in the classic model. In both cases, the classic model reached a stable equilibrium. The less efficient parasitoid with $a = 0.02$ (Figure 3.7.i), reached an equilibrium with a higher host density than the one reached by the more efficient parasitoid with $a = 0.025$ (Figure 3.7.ii), (126.1 and 105.6 respectively). One interpretation is that biological control by the parasitoids with lower search efficiency was aided by the female-biasing behaviour.

The arcing pattern appeared in the three dimensional plots, indicating that the populations are behaving in an almost identical way within each arc, due to the counterbalancing attributes of the sex ratio biasing behaviour and virginity discussed above. The most extreme values of sensitivity to competition and male mating efficacy, i.e. low (γ) and (λ), respectively, produced increases in host population levels. This was due to the effects of virginity becoming too strong, thus reducing the parasitoid efficacy as a biological control agent, or causing the parasitoid to become extinct.

3.5 Discussion

3.5.1 Biological control

An ideal biological control agent would greatly reduce host densities, but also maintain stable, low oscillatory population dynamics. Much of the theoretical work on biological control, especially concerning the NB model, has focussed almost exclusively on finding parasitoid-induced stability (Mills and Getz 1996). Other NB-based models have found, however, that stability comes with the trade-off of weaker host suppression (Murdoch and Stewartoaten 1989; Mills and Getz 1996; Singh et al. 2009). Although the

female-biased sex ratios in the model tended to reduce stability overall, there were many cases in which the model remained stable, while showing an improvement in host suppression. The regions that had the strongest improvements in host suppression, however, were the chaotic ones. In addition, with higher parasitoid search efficiency, many regions with increased chaos also had weaker host suppression or parasitoid extinction, as seen in Figure 3.7.ii and Figure 3.3.vi. Since Hassell and Varley (1969) added mutual interference to a NB-based model, several parasitoid-host models have incorporated interference where parasitoid efficacy decreases as competition for hosts increases, which in turn stabilizes dynamics. This functional response is to be expected in most cases, but it is in strong contrast to my model, since as competition for hosts became more intense, more females were produced and the parasitoids became more efficient as a consequence. A difference, however, is that the increasing proportion of females is cross-generational. Adding parasitoid interference would help stabilize my model by decreasing the amount of sex ratio bias. A decrease in efficacy at high parasitoid densities would decrease the frequency of superparasitism and thus decrease the sex ratio biasing behaviour.

Another essential aspect of classical biological control is establishment in a new area. A large part of establishment success is due to the initial densities of both the host and the parasitoid (Xiao and Tang 2008). I have shown here that sex ratio shifts in response to competition and virginity can greatly affect the stability of the system, sometimes leading to extinction, and thus could have strong effects on establishment success. Competition in a new area, however, should be very low, so the particular sex ratio shifting behaviour I have examined is unlikely to act on an establishing population.

Virginity because of difficulties in mate-finding, rather than because of a female-biased sex ratio, is more likely to be a factor that reduces the establishment success of a parasitoid (Hopper and Roush 1993).

3.5.2 Other work on sex ratios

There have been a few examples of theoretical work within the Nicholson-Bailey context on how competition between adults affects sex ratio. Hassell et al. (1983) examined three forms of density-dependent sex ratios. The first was purely a function of the size of the adult female population, the second depended on the ratio of females to hosts, and the third depended on the frequency of encounters with parasitized and unparasitized hosts. Their models looked at the opposite sex ratio shift, with populations becoming male biased as competition increased. Much like my model, their dynamics were highly dependent on the sensitivity to competition. Producing more males acted to stabilize their model, just as producing more females tended to destabilize mine. Increasing the proportion of males also, and not surprisingly, increased host densities. The third approach by Hassell et al. (1983) contrasts against mine, since frequency of superparasitism was also my measure of competition. In their models males emerged from superparasitized hosts, and females from singly parasitized hosts. This is the case of a heteronomous parasitoid, where the parasitoids have less control over their sex ratio. They found this to be strongly stabilizing, as when competition became more intense, the proportion of females in the next generation would decrease. Although heteronomous parasitoids have a fairly unique reproductive strategy, Hassell et al. (1983) point out that they have produced some of the most successful biological control interactions, and this strategy alone was enough to stabilize their model.

Aside from female biased sex ratio shifts due to the effects of local mate competition, most phenotypically plastic sex ratio shifts by parasitoids explored in the literature have been those involving a male shift. Since male offspring can survive on lower quality hosts (King 1993), one would expect more male offspring to be produced when there is an increase in superparasitism in a population, as previously parasitized hosts can usually be considered to be of lower quality. The sex ratio behaviour that I added in my model is thus quite novel, and little theoretical work has been done exploring this type of shift. Using a two-parasitoid, one host model, Kaitala and Getz (1992) showed that one species producing more females in response to competition will enhance its competitiveness, and as in my model, this will cause destabilizing effects, which can lead to extinction. Their model also kept track of males, though no effects of virgin oviposition were explored. They found that an inferior competitor could coexist with a superior one by biasing its sex ratio towards females. This agrees with my finding that increased female-biasing behaviour can produce similar dynamics to increasing parasitoid search efficiency (a) or conversion efficiency (c).

3.5.3   Virginity

When removing the female biasing behaviour in response to competition ($\gamma \rightarrow \infty$) the mated females simply produce offspring with the baseline sex ratio (σ) and virgins produce all-male offspring. I used this to examine the effects of virgin oviposition on the population in the absence of any sex ratio biasing behaviour by mated females. Stability and host suppression in this case were affected by changes in the baseline sex ratio and by male mating efficacy. When considering a system where the classic model reaches a stable equilibrium, increasing the virginity levels in the population decreased the efficacy

of the parasitoid, thus increasing host population levels. When considering a more

chaotic system, however, such as that with R = 4 and a = 0.08, virginity in the population

stabilized the system, but only once it became extreme. For example, with λ = 0.1,

dynamics were stabilized (Figure 3.9), but both the levels of virginity and chaos in the

absence of virginity were quite extreme, and cases such as this are unlikely to exist in

nature. I simply provide this as an example to demonstrate how virginity can change

dynamics in a similar way as a decrease in parasitoid search efficiency, just as the female

biasing behaviour can act in the opposite way.

My model assumes no differences between mated and virgin females apart from

their progeny sex ratio. This is a fair assumption, as a virgin female with no further

mating possibilities will always be selected to oviposit (Godfray 1994). However, virgin

females may behave differently. For example, they have been shown to produce larger

clutch sizes when laying in a patch with a mated female (Abe et al. 2010). Virgin females

have also been shown to attack fewer hosts in a patch and spread out their offspring at a

greater rate than mated females, thus increasing mating opportunities for their sons

(Fauvergue et al. 2008). The study by Fauvergue, et al. (2008) examined a species in

which mating and oviposition periods were overlapping, and virgin females were quickly

mated. Virgin females spent longer being inactive in patches, which they speculated was

calling behaviour. They proposed virgin females face a trade-off: call for mates and

oviposit while remaining in a patch, or spread their male offspring to more patches to

increase their sons' mating opportunities. In a system where there are no further mating

opportunities, virgin females may further increase this dispersal behaviour. Parasitoids

such as *Trichomalus perfectus* whose hosts are patchily distributed (Dosdall et al. 2006),

may experience some mating near the natal patch soon after emergence, so a dispersed all-male progeny could provide higher fitness than a clumped one where brothers would compete for the same females. Differing behaviours between virgin and mated females such as these could influence parasitoid-host dynamics, as well as reduce the impact on population sex ratio that virgin females have. Further theoretical work on this could prove to be interesting, potentially incorporating aggregated hosts, which have also been looked at in a NB context (Hassell and May 1973; May 1978).

3.5.4   How does the model reflect reality?

In the previous chapter, it was shown that a strong female bias can occur on a local scale in response to increased competition. It remains to be shown how this might scale up to the population level. Biologically accurate values for sensitivity to competition (γ) and male mating efficacy (λ) will no doubt vary considerably among species, but determining the range of biologically realistic values for both parameters would be interesting. In panmictic-mating systems such as the one in this model, mated females should be selected to produce female-biased broods to compensate for the all-male broods produced by the virgin females (Godfray 1994). Mated females thus may have some ability to assess the level of virginity in the population and female-biasing behaviour may change in response to that as well as to competition intensity. An accurate, current assessment may be impossible most of the time, but cues from previously parasitized hosts or from male encounter rates could be used (Godfray 1994). Fauvergue et al. (1998), for example, found that the parasitoid *Aphelinus asychis* would alter its sex ratio based on time spent as a virgin. They found that the parasitoid would respond in a step-wise fashion, altering its offspring sex ratio toward more females when

they spent more than one day as virgins. Individuals may be able to assess virginity levels through cues from previously parasitized hosts or encounters with other adult females. Ode et al. (1997) studied how mated female parasitoids alter their behaviour in the presence of virgin and sperm-depleted males. Although their example was one of a gregarious parasitoid, it did not locally mate. They found that mated females consistently produced a female-biased sex ratio, and they did not alter their ratio when constrained females were present. They proposed that an accurate assessment of constrained oviposition is not always necessary, and that this female-biased sex ratio produced by unconstrained females represents the average sex ratio necessary to compensate for constrained oviposition over evolutionary time.

This chapter provides one of the first examples of how virginity can affect population dynamics. I have shown that sex ratio biasing behaviour and virginity can have strong effects on population dynamics and biological control. The plastic sex ratio of *Trichomalus perfectus* demonstrated in the previous chapter thus has the potential to produce very interesting dynamics. It remains to be seen if population-level sex ratio shifts, or biologically significant levels of virginity occur in this species, but further study on this, as well as on virginity and sex ratio shifts between generations of other species could prove to be an interesting area of research.
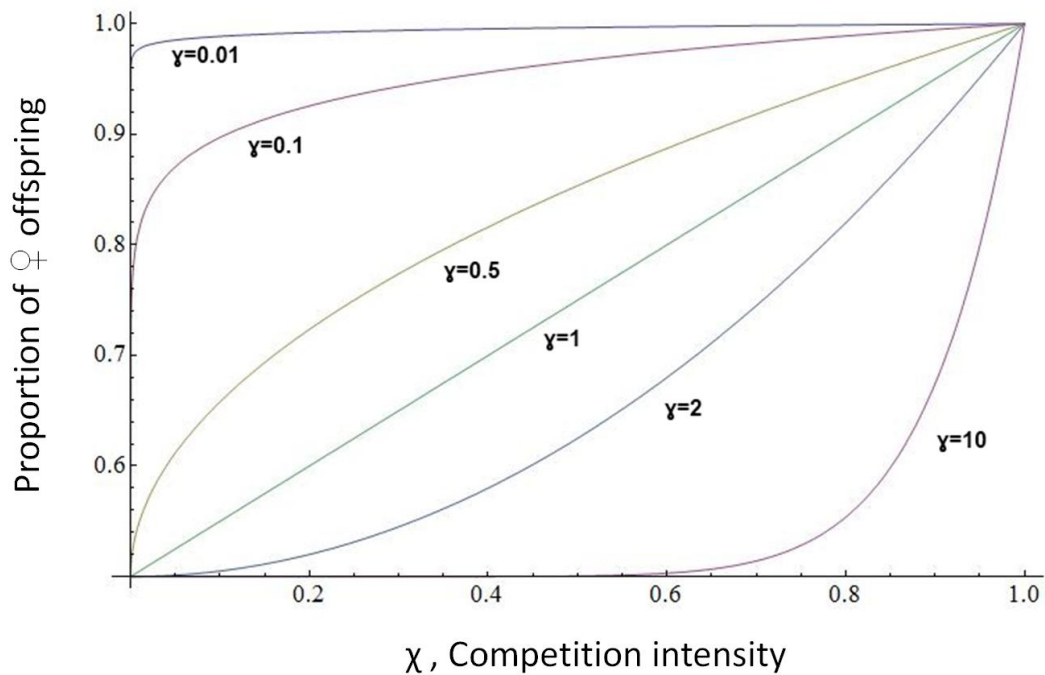
3.6 Figures

Figure 3.1 Response curves of mated female parasitoids with various sensitivities to competition (ɣ). The x-axis represents intensity of competition (χ), which is measured by the proportion of hosts that are superparasitized. The y-axis is the proportion of female offspring produced by the mated females. Lower ɣ values indicate a higher sensitivity to competition. As ɣ approaches zero, mated female parasitoids will alter their offspring sex ratio toward 100% females when any multiparasitism occurs in the habitat.
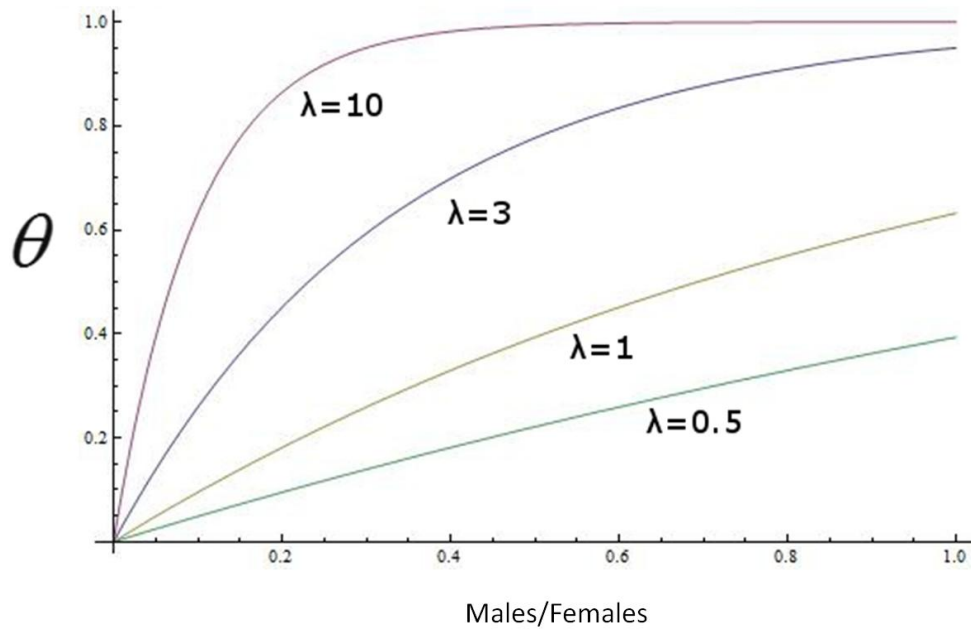
Figure 3.2 Curves with varying values of male mating efficacy (λ), showing the
proportion of female parasitoids that are successfully mated (θ) with given
population sex ratios. Increasing λ values can be biologically interpreted as
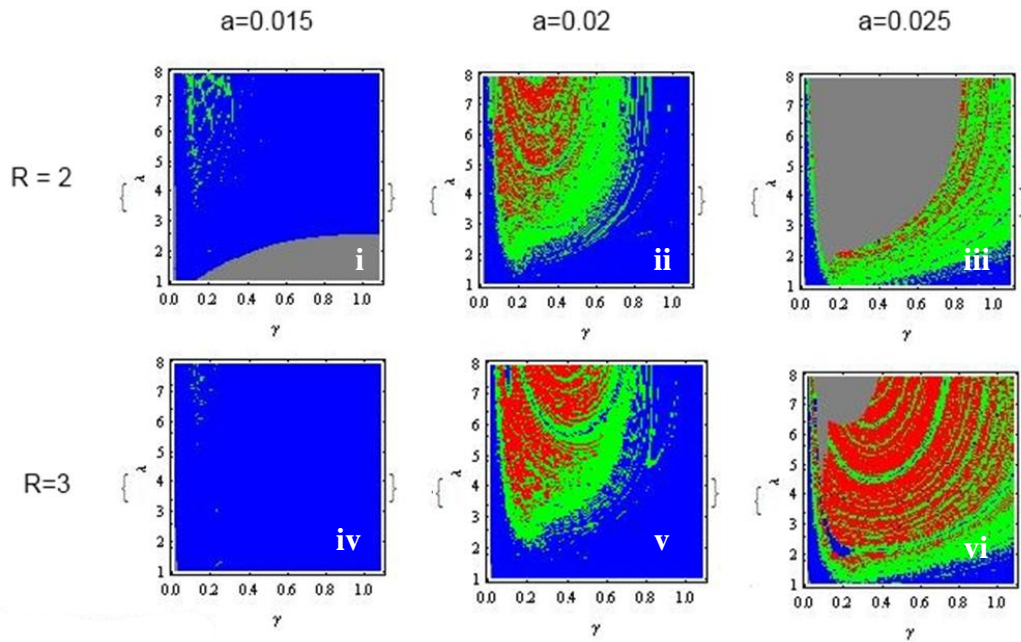an ability of males to mate with a greater number of females.

Figure 3.3   Behaviour of the model with various search efficiencies (*a*) and host
reproductive rates (R). Male mating efficacies (λ) are plotted on the vertical
axes of each graph against the sensitivities to competition (γ) values. Each
colour represents how the dynamics of the model behave for the given
parameters in the final 50 generations. Grey = parasitoid extinct, blue = stable
coexistence, green = erratic dynamics, red = chaos. With low search
efficiency, the parasitoids were only able to survive when there a higher
response to competition and male efficiency is high. Increasing search
efficiency values led to increasing regions of chaos and extinction. Increases
in *R* caused increased chaos, but in some cases reduced extinction caused by
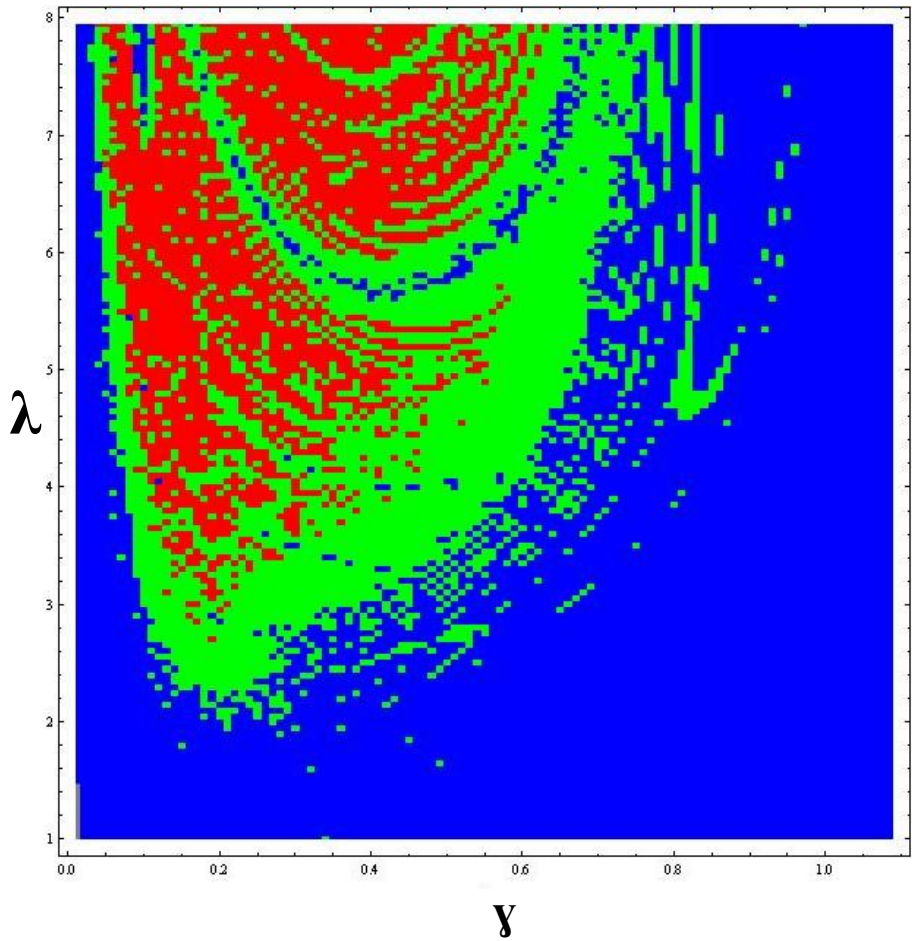the higher search efficiency of the parasitoid.

Figure 3.4 Behaviour of model with a=0.02, R=3, K=225. Each point on the graph represents a running of the model with specific value of sensitivity to competition (ɣ, x-axis) and male mating efficacy (λ, y-axis). Blue areas indicate stable coexistence. Red areas indicate chaotic coexistence, while green represents an intermediate level of stability between stable and chaotic. Grey areas indicate where the parasitoid has gone extinct.
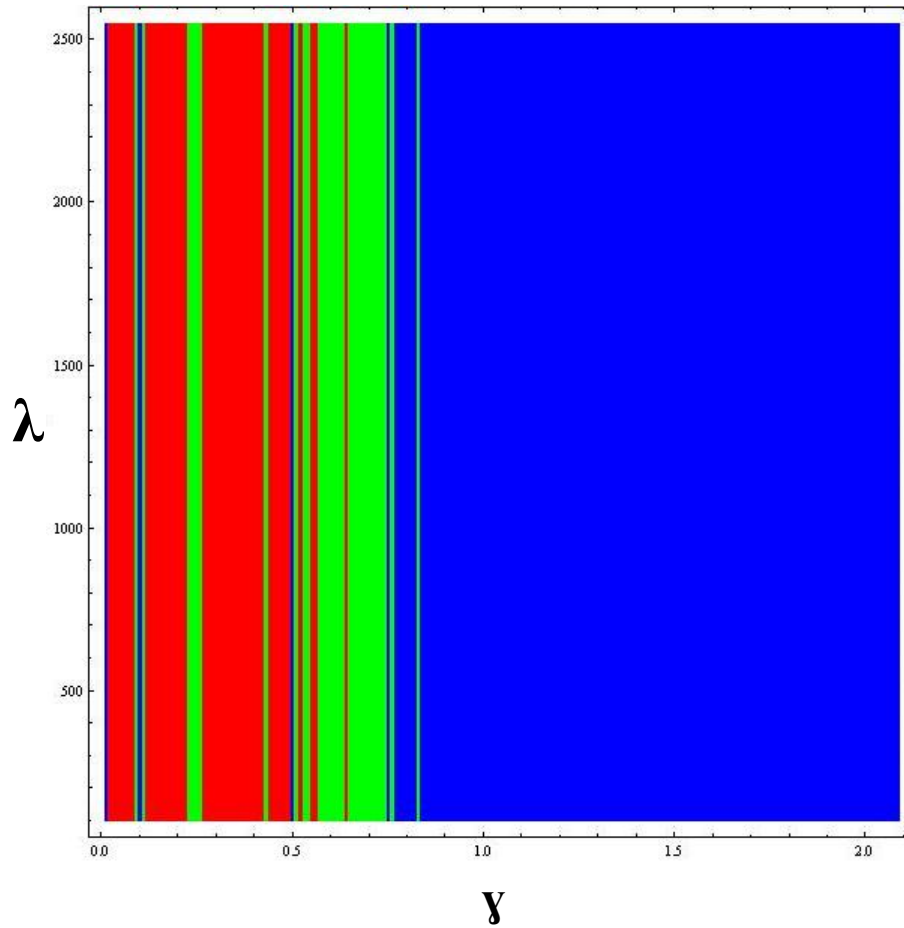
Figure 3.5 Dynamics of the model with very high values of male mating efficacy (λ). With no virginity in the system, increased sensitivity to competition (lower values of ɣ) led to increased chaos in the system.
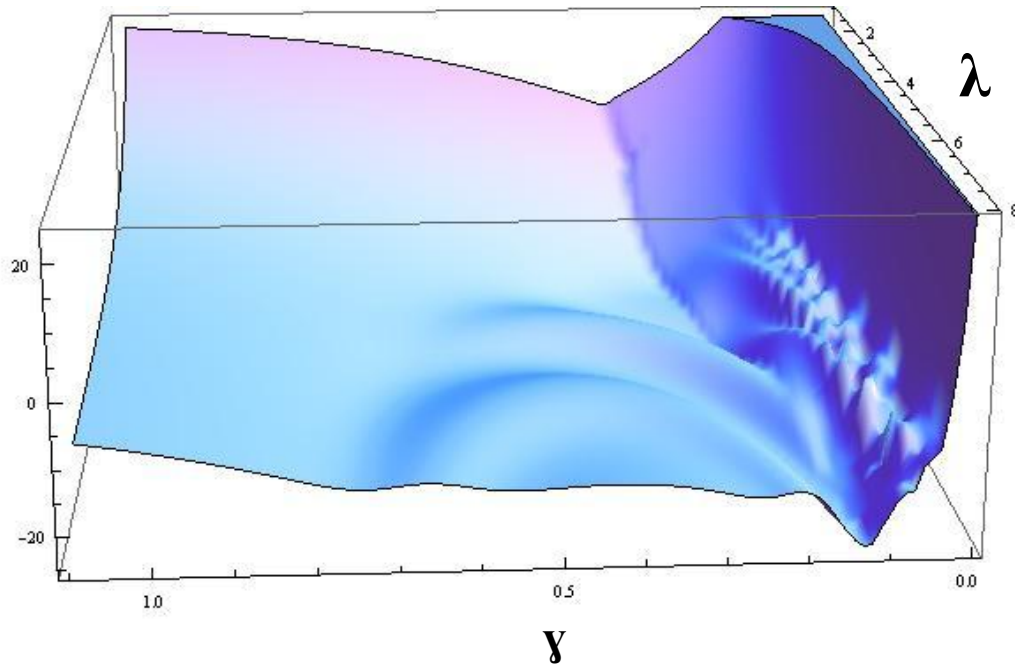
Figure 3.6 Three dimensional plot of host suppression in the new model with search efficiency, $a = 0.02$, and host reproductive rate, $R = 3$. Host suppression levels are on the vertical axis, which were measured by comparing the mean host population in the final 50 generations of the model when compared to the equilibrium density of the classic Beddington et al. (1978) model. The zero plane on the z-axis represents the equilibrium density of the Beddington et al. (1978) model, such that any instances below it indicate that the host population in the new model was kept at a lower density than the classic model, and any instances above the zero plane indicate a higher host density in the new model. The most varied areas in terms of host suppression differences were with low ɣ values, where response to competition was the most intense. In this case, there was a distinct trend toward a lower host population with increasing response to competition, as long as male efficacy (λ) remained high enough. With lower male efficacies, there was a dampening effect on the sex ratio produced, as the males can no longer fertilize an increasingly female-biased population. As λ decreases, there is a general increase in mean host populations.
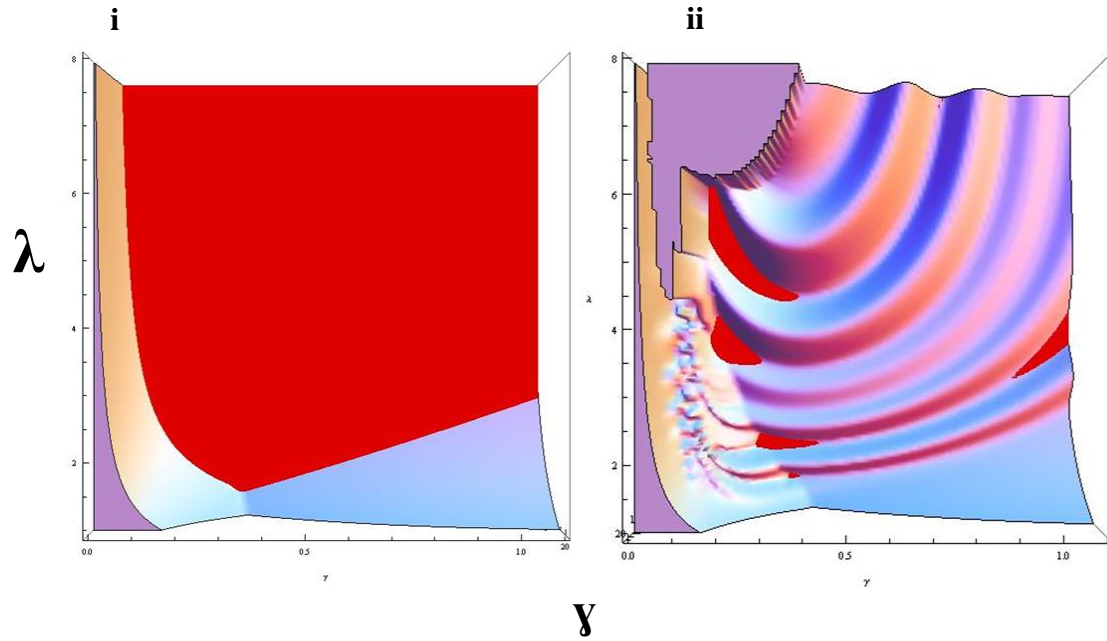
Figure 3.7 Side-by-side three-dimensional plots of two versions of the model. Both models differ only in the search efficiency of the parasitoid, where a = 0.02 (left) and a= 0.025 (right). The z-axis represents the change in mean host population relative to the base Beddington et al. (1978) model. Instances where z-values go below zero are coloured in red, and represent where the new model maintained host densities at a lower level than the base Beddington et al. (1978) model.
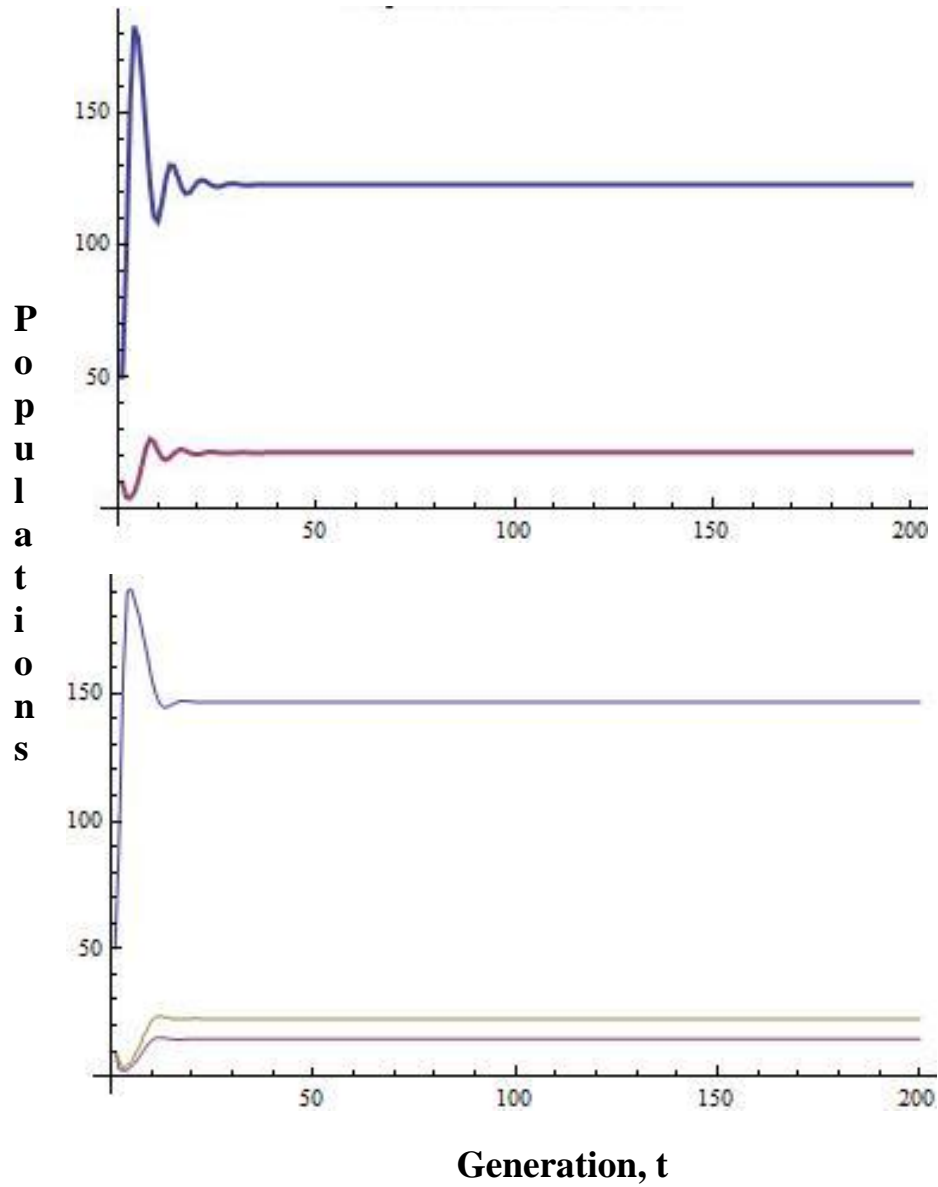
Figure 3.8 Comparison of a stable version of the classic model (top) and my model (bottom) showing the effects of virginity in the absence of sensitivity to competition (γ=10000). Both models had the same base parameters (a=0.02, R = 3). Virginity in this case decreased parasitoid efficacy as host population levels increased.

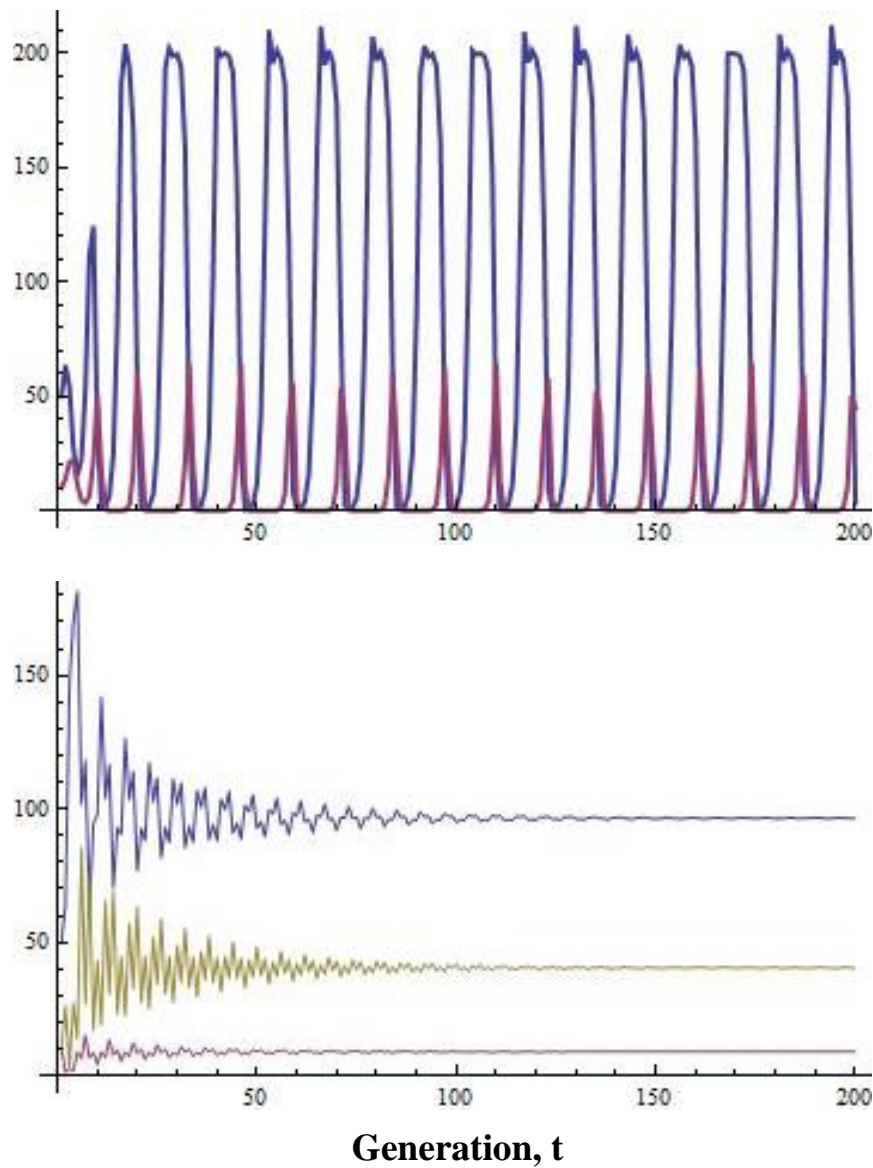Figure 3.9 Comparison of one iteration of the base Beddington et al. (1978) model (top) and my model (bottom) with an extreme case where males are highly ineffective (λ=0.1) and females do not respond to competition (ɣ=10000). Both models have the same parameter values, $a = 0.8$ and $R = 4$. High levels of virginity in my model caused the populations to become stable when compared to the base model.

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4: Conclusion

4.1 Conclusion

The purpose of my thesis was to explore the effects of competition among parasitoids exploiting a common host. Using the parasitoid guild of the cabbage seedpod weevil, I set out to answer questions regarding biological control, and examined some of the mechanisms behind competition in these species. I determined that a single species introduction for classical biological control of the cabbage seedpod weevil should be favoured over a two-species introduction. Although I could not detect any antagonistic effects on host suppression when two species were present, the safest, and possibly the most effective approach, would be a single species introduction of *T. perfectus.* Based on my experiments, neither species proved to be superior in its ability to attack hosts. Although their host suppression abilities are likely not identical, the two species have been found to produce similar mortalities of CSW in the field (Haye et al. 2010). From a practical standpoint, either *M. morys* or *T. perfectus* could thus provide a good level of host suppression if introduced into Canada. There are, of course, numerous other factors to be considered when choosing an ideal biological control candidate, but of key importance is the fact that *T. perfectus* is already present in Canada (Mason et al. In Press).

Through my experiments, I have revealed some interesting outcomes resulting from inter- and intraspecific competition between these species. The mechanisms behind larval competition in these species proved to be a very interesting. Although neither

species appeared to have a competitive advantage over the other at the larval level, I did find that the first instars of both species were highly aggressive and quickly killed (or were killed-by) any other con- or heterospecific competitors they encountered on the host. In addition, as there is no previous record of the development time of *M. morys*, the data reported in this thesis could prove useful for further research with this study system.

Possibly my most interesting finding was the sex ratio shift I observed in *T. perfectus*. I found that *T. perfectus* produced a female-biased secondary sex ratio when faced with a conspecific competitor, and an unbiased sex ratio when faced with a heterospecific. This shift was particularly interesting because it was a female bias that was neither caused by the effects of Local Mate Competition, nor was it an effect of host size. Although the specific mechanisms driving the sex ratio shift are unknown, the potential population-level effects of this phenomenon inspired Chapter 3 of my thesis. My model incorporated female-biased sex ratio shifts in response to intraspecific competition, demonstrating strong effects on biological control. An increasing female bias produced similar effects to an increase in parasitoid search efficiency – often improving host suppression, but also increasing the amount of chaos in the parasitoid-host dynamics. This was the first model of its kind to examine the effects of virginity on sex ratios caused by a shortage of males.

It has been established that sex ratios in *T. perfectus* are affected by host size (Murchie and Williams 1998). My work clearly demonstrates that other factors are also capable of impacting the sex ratios produced by this species. Investigating the mechanisms by which these factors influence sex ratios will be an interesting area for

further research. As *T. perfectus* has already been introduced into Canada (Mason et al.

In Press) and is likely to spread, greater understanding of how sex ratios are determined

in this species could prove profitable from a biological control perspective. Finally, such

work would likely provoke many useful theoretical contributions to the literature on sex

ratios in the parasitic Hymenoptera.

4.2   List of references

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