PHENOTYPIC PLASTICITY OF *TRICHOGRAamma MINUTUM* RILEY (HYMENOPTERA: TRICHOGRAmmMATIDAE) AND ITS IMPLICATIONS FOR MASS REARING

by

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A thesis submitted in conformity with the requirements for the degree of Master's of Science in Zoology

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Phenotypic plasticity of *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae) and its implications for mass rearing

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Abstract

Phenotypic plasticity of *Trichogramma minutum* was estimated to understand how and why the quality attributes of parasitoids vary under different environmental conditions. Parasitoid size, longevity and fecundity showed high plasticity when parasitoids were switched from a target host egg (spruce budworm) to a factitious host egg (flour moth). Arrhenotokous and thelytokous *T. minutum* reared at 15, 20 and 25°C showed higher plasticity for longevity and fecundity in both populations, while emergence and sex ratio were less plastic. Arrhenotokous *Trichogramma* were 2 to 5 times more plastic for any given trait when compared to thelytokous populations. Variance components demonstrated that rearing host and temperature caused >90% of the observed variation. The results are discussed further in terms of their ecological/evolutionary significance and implications for mass rearing of the parasitoids.
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To Rajumma, Gautham and Aishy

For all they have been and

For all they will be
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Chapter One: INTRODUCTION

Management of Canada’s forests has evolved through past decades from managing for timber alone to managing for all values of the forest. With the introduction of the Crown Forest Sustainability Act in 1995, forests are managed according to the principle of sustainability: that is to keep the forests large, healthy, diverse and productive (OMNR Annual Report, 1995/1996). Insect pests are considered a major threat to the health and vigour of our forests and they cause levels of damage that are considered unacceptable in the sustained management of the forests. Forest pest management involves implementation of various control measures like cultural practices and chemical control. Although chemical control measures play a major role in controlling the insect pests, public concern about the safety and the broader environmental impact of the insecticides has led to use of alternative approaches such as biological control.

Biological control agents form an important component in pest management strategies for plant protection against various agricultural and forest insect pests. Egg parasitoids belonging to the genus *Trichogramma* are the most significant biological control agents of lepidopterous pests in corn, cotton, sugarcane, fruits trees, vegetables and pines (Smith, 1996). *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae) has been suggested as a potential biological control agent in the control of the eastern spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae) through inundative releases in Canada (Smith et al., 1990). In this approach, high quality populations of *Trichogramma* are selected for mass rearing based on ecologically important attributes of parasitism such as high fecundity, longevity, sex ratio
(％ female), emergence, host preference, host searching activity and tolerance for the local weather conditions (van Lanteren, 1991; Bigler, 1994; Smith, 1996).

Research focused on parasitoid quality (= fitness of the parasitoids for biological control) indicates that quality attributes vary depending on the environmental conditions under which the parasitoids are mass reared (Calvin et al., 1984; Pak, 1986; Bai et al., 1992 & 1995; Bourchier et al., 1993 & 1994; Hassan, 1994; Wang and Smith, 1996). Such variation has been shown to affect the parasitoid’s efficiency in controlling pest populations (Bai et al., 1995; Smith, 1996). It is unclear how environmental factors influence variation in parasitoid quality or how they contribute to the success and survival of parasitoids upon release. Therefore, this thesis work was carried out to expand our understanding about the variation in parasitoid quality for mass reared egg parasitoids. *T. minutum* was used as a model because of its short life cycle, small size, its ease of rearing and the wealth of information available.

Studies from other organisms have showed that phenotypic variation could arise from: 1) genetic differences among individuals (genotypic); 2) environmentally induced phenotypic change (phenotypic plasticity); and 3) developmental noise (Via, 1994; Yampolsky and Scheiner, 1994). Among parasitoids, genetic variation has received considerable attention from researchers in recent years (Chassain and Bouletreau, 1991; Limburg and Pak, 1991; Antolin, 1992; Wajnberg, 1994). Although, there is extensive information available about the effects of environmental factors on parasitoid quality (Calvin et al., 1984; Pak, 1986; Bai et al., 1992 & 1995; Bourchier et al., 1993 & 1994; Wang and Smith, 1996), this work has focussed mainly on what the changes mean for the applied use of the parasitoids rather than exploring the basis of this variation.
Phenotypic plasticity is a simple and efficient way of examining the basis of environmentally induced variation because it allows for the precise estimation of variation, as well as elucidates the nature of the environmental effects on variation in quality. Phenotypic plasticity is defined as the change in the expression of a phenotype caused only by a change in the environment (Scheiner, 1993; Via, 1994). Studies on plasticity have been numerous in recent years ranging from morphological to biochemical, with morphological plasticity being the most common (Via, 1994). The increased interest also shows the importance of phenotypic plasticity in the evolution of environmentally induced changes of traits (Gotthard and Nylin, 1995).

Studies on phenotypic plasticity involving Trichogramma have been very limited (Kaiser et al., 1989; Liu, 1998). Kaiser et al. (1989) concentrated on plasticity of ovipositional behaviour in T. pretiosum Riley, whereas Liu (1998) has studied the plasticity of life history traits in arrhenotokous (males arise from unfertilized eggs) T. minutum. The recent discovery of thelytoky (females arise from unfertilized eggs=parthenogenesis) in T. minutum (Wang, 1994) allows for the estimation of how plasticity varies between the two reproductive forms. Another advantage of using the thelytokous population is that it consists of genetically identical individuals, and so it should be possible to precisely estimate the amount of variation caused by the environment. Besides, information on plasticity would be useful in choosing suitable populations of parasitoids for commercial use. For mass rearing, this type of study would provide insight into whether quality traits can be improved by manipulating rearing conditions such as host and temperature.
Objectives

The overall objective of the present study was to examine the basis and implications of phenotypic variation in the egg parasitoid complex of *T. minutum* more specifically, to measure the extent to which key quality characters are phenotypically plastic. The second objective was to compare the differences in plasticity for quality traits between arrhenotokous and thelytokous populations of *T. minutum*.

This thesis is organized into five chapters, with Chapter One being a general introduction. Chapter Two provides background information, a detailed review of past research on the effects of environmental factors on quality attributes in *Trichogramma* as well as a review of literature on phenotypic plasticity and how it is affected by factors like temperature, photoperiod and host or diet. Chapter Three examines the effect of switching the rearing hosts on the plasticity of quality traits for an arrhenotokous population of *T. minutum*, while Chapter Four compares the plasticity of quality traits for arrhenotokous and thelytokous populations of *T. minutum* under different rearing temperatures. A summary of these findings, along with a general discussion on implications for mass rearing is in Chapter Five. One overall bibliography for the entire thesis can be found at the end. Because Chapters Three and Four were written in the format of a journal article, there may be some overlap in background information and methods. The Results and Discussion sections, however, are distinct for each of the chapters.
Chapter Two: Background information

This chapter is divided into two sections: in the first section I discuss the literature about the influence of rearing conditions on parasitoid quality under laboratory conditions. In the second part, I review the literature on the basics about plasticity, the various environmental factors affecting plasticity and how the reaction norms change under these conditions.

Environmental factors and parasitoid quality attributes

The success of any inundative program depends on two critical factors, the environmental conditions into which the parasitoids are released and the quality of the parasitoids being released (Bourchier and Smith, 1996). The quality of the parasitoids partially depends on the conditions under which they are mass reared. For Trichogramma, the influence of environmental factors (rearing host, temperature, photoperiod, humidity and adult nutrition) on quality attributes has been studied extensively in the laboratory. In the following review, I discuss each of these factors in their order of importance to parasitoid quality.

Effect of host characteristics

Host species and size: In mass rearing, the choice of rearing host depends ultimately on the size of eggs produced. Studies addressing the effect of host species on parasitoid quality have looked at host egg size because this influences the size of the emerging parasitoid. In general, the size of the eggs produced by the natural or target host is larger
than the factitious host (Smith, 1996). In North America, eggs of the Angoumois grain moth, *Sitotroga cerealella* Olivier or the Mediterranean flour moth, *Ephestia kuehniella* Zeller have been used as factitious hosts for mass rearing *Trichogramma*. Factitious hosts are used because they reduce the cost of rearing, however the important concern is the quality of parasitoids produced relative to those emerging from the natural host (Bigler, 1988).

Researchers have measured the size of parasitoids emerging from natural and factitious hosts, because size has been considered as a measure of parasitoid quality. For example, the size of *Trichogramma* emerging from hosts like *C. fumiferana*, *Helicoverpa zea* (Boddie), *Manduca sexta* (Johanssen) and *Diatrea rufenscens* Box, is larger than those emerging from factitious hosts such as *E. kuehniella* and *S. cerealella* (Bai et al., 1992; Bourchier et al., 1993; Bai et al., 1995; Greenberg et al., 1998; Monje et al., 1999). A positive correlation also exists between the size of the parasitoid and other quality attributes. Larger parasitoids reared from natural hosts are found to be more fecund and longer living than smaller parasitoids reared from factitious hosts (Smith and Hubbes, 1986; Hohmann et al., 1988; Bai et al., 1992; Bourchier et al., 1993; Bai et al., 1995). In addition, an increase in the % parasitism has been noted for larger parasitoids (Greenberg et al., 1998).

Host species also influences host preference and acceptance behaviour by *Trichogramma*. Continuous rearing of *T. maidis* Pintureau & Voegele on *Ephestia* lowered parasitism on *Ostrinia nubilalis* (Hbn.) because of low host acceptance or host suitability, and host acceptance decreased with an increasing number of generations reared on *E. kuehniella* (van Bergeijk et al., 1989). Bourchier et al. (1993) have observed
a similar result, where *T. minutum* reared on spruce budworm did not realize their full potential fecundity due to low host acceptance for *E. kuehniella* eggs. To prevent the negative impact of a factitious host on host preference, measures such as switching the rearing host have been carried out in countries like Switzerland (Bigler, 1986). However, the findings of Kaiser *et al.* (1989) suggest that such measures are always not needed. They reported that the preference for *O. nubilalis* by *T. pretiosum* was not affected due to continuous rearing on *E. kuehniella* because *T. pretiosum* had an innate preference for *O. nubilalis* even after 100 generations on *E. kuehniella*.

**Host switching:** Host switching is a fundamental component of mass rearing programs for *Trichogramma*. Because of the ease of rearing large numbers of host species, parasitoids are often mass reared on a relatively small factitious host such as *E. kuehniella* and then released to attack large hosts like *C. fumiferana*. Bourchier *et al.* (1994) have observed that *T. minutum* switched from rearing on the natural host to rearing on a factitious host have lower fecundity. Similarly Corrigan and Laing (1994) reported a significant reduction in fecundity, longevity, % emergence and sex ratio of *T. minutum* when they were switched from *C. fumiferana* to *E. kuehniella*.

**Host age:** Host age is another factor affecting host preference and acceptance behaviour of parasitoids. In general, host age has been reported to affect host attack rate, oviposition, rate of development, mortality (Pak, 1986), parasitism, and fecundity (Calvin and Losey, 1990; Reznik and Umarova, 1990; Monje *et al.*, 1999). When given a choice between young and old host eggs, *Trichogramma* spp. prefer young hosts (Pak, 1986;
Calvin and Losey, 1990; Reznik and Umarova, 1990; Monje et al., 1999). Rejection of older eggs has been attributed to the inhibition of oviposition because of physiological changes in older eggs (Reznik and Umarova, 1985), sclerotization of the head capsule (Pak, 1986), or to the rotation of the host embryo (Reznik and Umarova, 1990). On the contrary, Pak et al. (1986) found females of T. maidis, T. brassicae Bezd. and T. evanescens Westwood generally prefer both young and old eggs of M. brassicae L., Pieris brassicae L. and P. rapae L. equally, only oviposition was lower in 3 day-old M. brassicae eggs than 1 day-old eggs.

**Effect of temperature**

*Trichogramma* are poikilothermic and their biology depends on ambient temperature fluctuations. Most *Trichogramma* spp. require a minimum threshold temperature of 10 to 13°C for their development (Calvin et al., 1984; Lawrence et al., 1985; Grille and Basso, 1994; Wang and Smith, 1996). Parasitoid developmental rate, longevity, fecundity, parasitic activity and flight activity have been found to be inversely related to rearing temperature, with a range of 20 to 30°C being reported as the optimum for all of these parameters (Pak and Oatman, 1982; Calvin et al., 1984; Harrison et al., 1985; Lawrence et al., 1985; Pak and van Heiningen, 1985; Gross, 1988; Forsse et al., 1992; Babi, 1994; Garcia and Tavares, 1994; Parra and Sales, 1994; Wang and Smith, 1996). Temperatures ≥ 32°C have been reported to: 1) increase the % of females in *T. brevicapillum* Pinto & Platner (Pak and Oatman, 1982); 2) induce the development of brachypterous wings in *T. pretiosum* (Gross, 1988); 3) increase the % of males; and 4) cause mortality in *T. galloi* Zucchi (Para and Sales, 1994). Low temperatures have been
shown to prolong adult longevity in many species: this increase being attributed either to lower metabolic activity (Para et al., 1990) or a difference in the mode of reproduction (Wang and Smith, 1996). In species like *T. minutum*, temperatures below 17°C have been shown to produce a high % of females and reduce the emergence rate (Smith *et al.*, 1986; Wang and Smith, 1996).

**Effect of photoperiod**

Although photoperiod is not commonly associated with changes in parasitoid development, a few studies have examined the effect of photoperiod in *Trichogramma*. The response of *Trichogramma* to photoperiod varies with species; some species exhibit relatively intense, photoperiodically controlled diapause, while other species or strains completely lack a response to photoperiod (Laing and Corrigan, 1995). Calvin *et al.* (1984) have shown an increase in developmental time under longer day lengths and a decrease in adult female longevity under shorter day lengths in *T. pretiosum*. Fecundity and sex ratio were found to be unaffected by photoperiod in the same study. Short day lengths (12 L : 12 D) during the parental generation, in combination with short day lengths and low temperature (15°C) during the offspring generation, have been shown to induce diapause in the offspring generation itself for six species of *Trichogramma* (Zaslavski and Umarova, 1990). Laing and Corrigan (1995), in their attempt to induce diapause in *T. minutum*, reported that neither photoperiod nor temperature could induce diapause in this species. They concluded that even though diapause was an important aspect that could keep the parasitoids under dormant conditions during off-seasons in
mass rearing facilities, it could only be induced in species like *T. minutum* by a combination of host species, photoperiod and low temperature.

**Effect of humidity**

*Trichogramma* are internal parasitoids and the developing stages are well protected from desiccation by the host egg chorion hence the influence of humidity has been studied only to a limited extent. In general, humidity levels of 60 to 80 % are thought to be optimal for longevity, fecundity, and emergence (Calvin et al., 1984; Gross, 1988; Leatemia et al., 1995), whereas relative humidities in the range of 20 to 40 % have been shown to increase the developmental time (Calvin et al., 1984) and reduce the emergence rate (Gross, 1988) in species like *T. pretiosum*.

**Effect of adult nutrition**

The availability of food sources to adult parasitoids such as honey and fructose-glucose solutions has been reported to improve their quality during mass rearing. Longevity is the trait most influenced by adult nutrition. When compared to un-fed parasitoids, honey- (or sucrose-) fed individuals have been shown to live 5 to 21 times longer than un-fed individuals (Yu et al., 1984; Smith et al., 1986; Hohmann et al., 1988; Bai et al., 1992; Leatemia et al., 1995; McDougall and Mills, 1997). Similarly honey-fed females were found to be more fecund and to produce more offspring than un-fed females (Yu et al., 1984; Hohmann et al., 1988; Bai et al., 1992; Leatemia et al., 1995), although the offspring sex ratio was more male-biased for those living longer and producing more individuals (Leatemia et al., 1995). Forsse et al. (1992) demonstrated an increase in
flight propensity for female *T. minutum* in the presence of honey, while the timing of flight was unaffected.

**Environmental factors and phenotypic plasticity**

The literature on phenotypic plasticity is extensive, and compiling all such information in this section is neither possible nor necessary for the purpose of my thesis. I have reviewed only that literature pertaining to fitness traits because the characters that constitute quality traits for *Trichogramma* are considered fitness traits in other organisms.

Until recently, little effort has been shown to explain the effect of environment on phenotype and their evolutionary consequences. However, geneticists and evolutionists now understand that the environment is an agent for development and that it influences the range of phenotypes produced by a given genotype. By affecting the phenotypes expressed, the environment influences which one is exposed to selection and modified during evolution (West Eberhard, 1989). Because of this, there has been an increased interest in phenotypic plasticity in recent years. The definition of phenotypic plasticity varies according to author and school of thought, but the definition given by Via (1994) is simple and clear. According to her, "for a given genotype a change in the phenotype caused only by a change in the environment is defined as phenotypic plasticity". This change in phenotype can be behavioural, morphological, physiological or biochemical (West Eberhard, 1989; Via, 1994).

The plastic response to an environmental cue has five attributes: the *amount* of plasticity (magnitude of the response - large or small), the *pattern* (shape of the response - linear or non-linear), *rapidity* (speed of the response- fast physiological changes versus
slow developmental changes), reversibility (capacity to switch between alternative states – the photosynthetic rate of a leaf is reversible), and competence (ability of the developmental system to respond to environmental cue during a particular time ‘window’ in the ontogeny) (Schlichting and Pigliucci, 1998). It should be noted that even though environmental cues trigger phenotypic differences, the ability to respond to these cues is genetically based and the extent of that response can evolve under natural selection (Via, 1994). The genetic mechanism that influences the plastic response can either be allelic sensitivity (differential expression of alleles in different environments causing differences in phenotype) or gene regulation (regulatory loci causing other genes to be turned on or off in a particular environment) (Via et al., 1995; Schlichting and Pigliucci, 1998).

Phenotypic plasticity can be studied by measuring the norm of reaction – which is the set of (possibly different) phenotypes produced by a single genotype across a range of environments (Via et al., 1995). Plasticity may be adaptive or non-adaptive and reaction norms (or rather a given aspect of it) may or may not qualify as adaptation to a given environment (Via et al., 1995; Nylin and Gotthard, 1998). There are two basic methods of quantifying plasticity: the character state approach and the polynomial approach. In the character state approach, the reaction norm for a particular character is modeled as the set of phenotypic values that would be expressed in each environment by a given genotype (Via, 1984; 1993). In the polynomial approach, the reaction norm is described by a response curve, with parameters for the intercept (a), the slope (b), and the curvature (c) (Scheiner, 1993; Schlichting and Pigliucci, 1998). Which approach to use for empirical studies should be based on the type of environments used, the type of data collected and the kinds of issues that one wishes to address (Via et al., 1995).
The study of phenotypic plasticity for fitness traits is in fact the study of plasticity for life history traits because it is those life history traits that determine how well an organism does in terms of development, reproduction and survival. Plasticity in life history traits may represent an adaptation to environmental variation (Gotthard and Nylin, 1995; Via et al., 1995; Nylin and Gotthard 1998). There is a significant body of information concerning environmental factors and how they affect life history (fitness) traits. The following section discusses them in their relative order of importance.

**Effect of Photoperiod on plasticity**

Photoperiod is the main environmental signal indicating the progression of the season and a cue to adjust the life cycle in ectotherms like insects (Nylin and Gotthard, 1998). Induction of diapause as a response to day lengths is perhaps the best-documented case of adaptive plasticity in insect life histories (Danks, 1994). However, seasonality may also favour plasticity in the development of insects growing late in the season (Leimar 1996; Nylin et al., 1996) and as a result, shorter developmental times may arise as adaptive plasticity in those insects. Photoperiod-induced reaction norms for developmental rates are well documented in several butterfly species, with short day lengths inducing fast growth and developmental rates in the larvae of *Polygonia c album* L. (Nylin, 1992), *Polyommatus icarus* Rottemburg (Leimar, 1996), *Lasiommata petroplitana* L. (Gotthard, 1998), *L. maera* L., and *Lopinga achine* L. (Gotthard et al., 1999).

The response to photoperiod may also depend on the individual species. For example, the reaction norm relating larval development to day length differs in *L. maera* and *L. achine* (Gotthard et al., 1999). Although these two butterfly larvae had a positive
reaction norm for short photoperiod before the autumn diapause, the response in spring changed after the diapause. In *L. maera*, the reaction norm for development and day length had a negative slope in spring, while in *L. achine* the reaction norm changed from positive to zero in spring. Photoperiod has a mixed effect on adult size. For instance, shorter day lengths did not affect the adult size in species like *P. icarus* (Leimar, 1996), *L. maera* and *L. achine* (Gotthard et al., 1999), but in *L. petropolitana* shorter larval times and high growth rates reduced the pupal size suggesting a trade-off between development time and size at pupation due to short photoperiod.

**Effect of temperature on plasticity**

Temperature and its changes (diurnal, nocturnal and seasonal) can have a major impact on the distribution and abundance of insects in time and space. As a general rule, increased temperature will result in higher growth rates and shorter developmental times and often reduced adult size in insects (Atkinson, 1994; Nylin and Gotthard, 1998). Support for this theory comes from several insect taxa. For example, the reaction norm relating developmental time and temperature is found to be negative in milkweed bug, *Oncopeltus fasciatus* Dallas, when the rearing temperature was changed from 23 to 27°C (Groeters and Dingle, 1988). Similar observations have been recorded in *B. safitza*, *B. anynana* (Brakefield and Reitsma, 1991), *D. melanogaster* L. (Worthen, 1996; Land et al., 1999) and *Callosobruchus maculatus* F. (Guntrip and Sibly, 1998). Also, a higher level of plasticity in developmental time might be expected to evolve in populations encountering more variable thermal environments. Worthen (1996), in a comparative study between tropical and temperate populations of *D. melanogaster*, reported a
significant ‘population x temperature’ interaction for developmental time indicating plasticity among populations. However, he noted that the populations from temperate to mid-latitude developed faster at 18 to 25°C than those from a tropical latitude, suggesting that populations from seasonally variable habitats are better adapted to thermally stressful environments than populations from more consistently warm climates.

Body size is another fitness trait that shows a plastic response to rearing temperatures in ectotherms, with larger sizes observed at cooler temperatures (Atkinson, 1994; Berrigan and Charnov, 1994). Although the measure of size varies with the type of organism studied and the type of analytical methods employed, plasticity has been reported in the size of several insect species regardless of the measure. Most studies have reported a monotonically decreasing reaction norm for size as a response to rearing temperature (Groeters and Dingle, 1988; Brakefield and Reitsma, 1991; David et al., 1994; Patridge et al., 1994; Delpuech et al., 1995; Noach et al., 1997; Nunny and Cheung, 1997; Loeschecke et al., 1999). But, there are a few exceptions to this rule of decreasing size with increasing temperature. Guntrip and Sibly (1998) detected no genotype x environment interaction for body weight in C. maculatus while the body weight increased with an increase in temperature. Morin et al. (1999) observed a convex reaction norm for thorax and wing length in D. melanogaster when the females were reared at 12 to 32°C meaning that the thorax and wing lengths were smaller at low and high temperatures and larger at intermediate temperatures.

Fecundity is a major determinant of female fitness and females raised at extremely low or high temperatures are typically less fecund than those raised at intermediate temperatures. Studies with T. minutum provide an example for this, where
the females reared at 20 °C had the highest fecundity compared to those reared at 15 or 25 °C (Liu, 1998). However, this generalization has been found to be lacking in many recent studies. The parasitic wasp, Muscidifurax raptor Kogan & Legner showed an increase in fecundity when raised at higher temperatures with no genotype x environment interaction between the strains of wasps and rearing temperatures (Antolin, 1992). Huey et al. (1995) observed that female D. melanogaster reared at 18 and 25°C produced more offspring at 25°C and found no evidence of an interaction between rearing temperature and fecundity. Similarly, Patridge et al. (1995) and Nunny and Cheung (1997) reported higher fecundity in D. melanogaster when the rearing and test temperatures were the same than when the rearing and test temperatures were different.

Reaction norms for sex ratio may result if the sex-determining mechanism of a species allows sex ratio to vary according to the environmental condition. Also, because sex ratio is partly determined by fecundity, plasticity and genetic variation for sex ratio may be limited by the plasticity and genetic variation available for fecundity (Antolin, 1992). Recent studies with parasitoids such as M. raptor (Antolin, 1992) and T. minutum (Liu, 1998) indicate that the sex ratio reaction norm can vary with rearing temperature. They can be linear and female-biased across the test temperatures.

Effect of diet and host species on plasticity

Most ectotherms show a reduced growth rate and delayed maturation at a small size under decreased or low food conditions (Berrigan and Charnov, 1994). Such plastic effects of diets may sometimes be purely maladaptive, as they directly affect the basic resources available for growth, maintenance and reproduction (Nylin and Gotthard,
Gebhardt and Stearns (1988 & 1993) reported longer larval developmental time and reduced size at emergence in *D. melanogaster* and *D. mercatorum* (L.) raised in a growth medium with a low yeast concentration. In addition, sexual dimorphism in developmental time was also noticed, with males developing faster than females at low yeast concentrations. The lycaenid butterfly, *Taraka hamada* Druce showed plasticity in developmental time and adult size when grown under limited supply of aphids, *Ceratocaruna japonica* Takahashi (Banno, 1990). In the lygeid bug, *O. faciatus*, the Sicilian and Italian populations responded differently to varying food levels (Dingle, 1992). The large-sized Italian population showed mal-adaptation when reared at low food level of 3-milkweed seeds/nymph from hatching to adult eclosion, while the small-sized Sicilian population did not show any adverse effect at all. *Aphis gossypii* Glover were significantly bigger when reared on cucumber and rock melon than when reared on cotton, broad beans and egg plants (Wool and Hales, 1997), suggesting that cucumber and rock melon were nutritionally better for growth than other host plants.

Not all insects show mal-adaptations under poor diet conditions. The grasshopper, *Melanoplus femurribrum* De Greer (Thompson, 1992 & 1999), and several sawfly species (Kause et al., 1999) show compensatory consumption under low quality diet to buffer larval growth rates and maintain normal size. When the grasshoppers were raised on low quality - hard plant diet (*Lolium perenne* L.) and good quality - soft plant diet (*Trifolium repens* L.), they enhanced their performance by increasing their consumption rate 37% on *L. perenne*. In addition, they developed larger head size and mandibles to improve feeding on *L. perenne*. The sawflies displayed behavioural plasticity in the form of dispersal from feeding sites. The late summer species,
*Priophorus pallies* Lepeletier, *Arge* spp. Schrank and *Dineura pullior* Schmidt & Walter responded to low quality birch foliage by increasing their consumption rate, while the early summer species, *Amauroneumatus amplus* Konow and *A. kemoevsis* Vikberg dispersed from feeding sites in order to find high quality leaves. This way they maintained stable growth rates on diets of variable quality.

Difference in diet also affects fecundity reaction norms. The long-winged water striders, *Gerris thoracicus* (Schummel), *G. odontogaster* (Zett.) and *G. lacustris* (L.), showed plasticity in reproductive allocation when maintained under low and high levels of food (Kaitala, 1991). Under low food levels, *G. lacustris* females had smaller egg production and the females traded-off longevity for fecundity, whereas *G. odontogaster* and *G. thoracicus* females only decreased their daily and total egg production, and did not trade off their longevity for fecundity. In the case of the milkweed bug, *O. faciatus*, when large and small bugs were reared from eclosion on 12, 18 and 24 seeds per adult, the large bugs were affected adversely and only a few individuals produced eggs at 12 and 18 seeds per adults, while the small bugs did not suffer any adverse effect (Dingle, 1992). In conclusion, food level reaction norms suggest that size is the primarily factor affected by diet quality and larger size may be selected against during low food levels and reduction in fecundity can be observed due to small size.
Chapter Three: Phenotypic plasticity of quality characters for

*Trichogramma minutum* Riley in response to host switching

INTRODUCTION

*Trichogramma minutum* is a hymenopteran egg parasitoid used in inundative releases for biological control of the eastern spruce budworm, *Choristoneura fumiferana* (Smith *et al.*, 1990). Although up to 80% success has been achieved in controlling the budworm in the past (Smith *et al.*, 1990), the results vary widely depending on the efficiency of the parasitoids released. The efficiency of *Trichogramma* in controlling pest populations is highly related to its quality (Bigler, 1991 & 1994). Quality is often defined as high fecundity, longevity, sex ratio (% female), emergence, host preference, host searching activity and tolerance for the local weather conditions (van Lanteren, 1991; Bigler, 1994; Smith, 1996).

Recent studies on parasitoid quality indicate that quality characters vary depending on the environmental conditions under which the parasitoids are reared (Bigler, 1991; Smith, 1996). Rearing host is one of the main environmental factors that has received attention. Host characteristics such as host species (Corrigan and Laing, 1994; Bai *et al.*, 1995), host size (Bai *et al.*, 1992; Bourchier *et al.*, 1993), switching between rearing hosts (Bourchier *et al.*, 1994), and reproductive modes (Wang and Smith, 1996) have all been shown to affect quality characters. Host switching is a fundamental component of mass rearing programs for *Trichogramma*. Because of the ease of rearing large numbers of host insects, parasitoids are often mass reared on a
relatively small factitious host egg, such as Mediterranean flour moth, and then released to attack a larger target host, such as spruce budworm (Bourchier et al., 1994). Host switching has been reported to significantly influence fecundity, host acceptance, longevity, emergence and % female offspring (Bourchier et al., 1994; Corrigan and Laing, 1994). These studies have examined the implications of phenotypic changes for applied aspects of pest control rather than how these variations arise and contribute to the success and survival of the parasitoid in the field.

Environmentally induced phenotypic variation is known as phenotypic plasticity (Scheiner, 1993; Via, 1994). Phenotypic plasticity is a simple and efficient way of examining the basis of environmentally induced phenotypic variations. In addition it allows the precise estimation of the extent, as well as the nature of environmental effects on quality variations. In this chapter I estimate the extent of plasticity in the quality traits of *T. minutum* when switched between two rearing hosts. In this case, the spruce budworm *C. fumiferana* (Lepidoptera: Tortricidae) and the Mediterranean flour moth, *E. kuehniella* (Lepidoptera: Pyralidae) are the hosts used. In addition, I estimate the variance components to examine the contribution by rearing host (environment) and genotype of the parasitoids (genotype) to the observed variation in quality traits and discuss the implications of these findings for the mass rearing of *Trichogramma*.

**MATERIALS AND METHODS**

**Parasitoid colony**

The experimental population of *T. minutum* originated from parasitized eggs of pine false webworm, *Acantholyda erythrocapala* L. (Hymenoptera: Acantholydae)
collected near Sprucedale, northern Ontario (79 °W, 45 °N) during 1995. They were reared for one generation on webworm and then switched to spruce budworm eggs at 20°C and 16 = 8 light = dark conditions. The parasitoids had completed approximately 60 generations before the experiment was initiated in the laboratory. The population size was maintained around 5,000 individuals in each generation and there was no overlapping of generations during the rearing process.

**Experimental procedure**

A sib-mating design was used to test the same genotype across different host environments (Fig. 3-1). In this method, half and full-sibs families were created by allowing 40 virgin females and 10 virgin males from the same cohort (< 2 h old) to mate at a ratio of 1 male = 4 females for two hours in the parental generation in small glass vials (3.5 x 1.5 cm). At the end of two hours, each mated female was placed in a small glass vial (3.5 x 1.5 cm) and was given an egg mass of budworm and flour moth eggs (approximately 100 eggs) for 24 h in a no-choice trial. The order in which budworm and flour moth were given was decided randomly so that the type of egg given did not bias oviposition. Flour moth eggs were chosen as the second host because of its ease in rearing, handling and it is the commercial host for *T. minutum*. A streak of 50 % honey solution was applied as a food source on the sides of the vial during the trial. Those parental females who were able to parasitize the eggs of both hosts were included for further observation; only 14 parental females (2 females/male) successfully parasitized both budworm and flour moth and this reduced the actual number of full sib families from 40 to 14.
Figure 3-1. Schematic representation of sib-mating design used for the arrhenotokous *Trichogramma minutum* in the host switching experiment. *F₀* = Parental generation and *F₁* = First filial generation.
The parasitized eggs were incubated at 20°C and 16:8 light:dark until the F₁ generation emerged. The number of emerged F₁ females per parental female varied from 3 to 15. The newly emerged F₁ males and females (sibs) were allowed to mate randomly with each other and mated F₁ females from each family were isolated into small glass vials and given either budworm or flour moth eggs. Each female was given an egg card (approximately 100 eggs) three times (day 0, day 1 and day 3) in the F₁ generation. A streak of 50 % honey solution was applied to the sides of the vial as food, and longevity was observed for each F₁ female. The parasitized budworm and flour moth eggs were incubated at the same conditions as the parental generation. Size of the right fore wing of each F₁ female was recorded as a measure of size. Data on the first-day fecundity (no. of progeny produced on the first day), total fecundity (total no. of progeny) and sex ratio (% females) of the progeny were recorded for the F₂ generation.

**Statistical Analyses**

Data on size, longevity, first-day fecundity, total fecundity and sex ratio were checked for normality (Shapira and Wilk’s method) and homogeneity of variance using Cochran’s T test (SAS, version 6.03, 1989). All data except sex ratio were normally distributed. Data on sex ratio were transformed using log transformation before any further analysis. Pearson correlation coefficients were estimated to check for the correlation between parasitoid size and other quality parameters (Table 3-1). Because
Table 3-1. Pearson correlation coefficients for quality traits in *Trichogramma minutum* reared on spruce budworm and Mediterranean flour moth eggs at 20°C.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Size</th>
<th>Longevity</th>
<th>First-day fecundity</th>
<th>Total fecundity</th>
<th>Sex ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>1.00</td>
<td>0.04 (0.68)*</td>
<td>-0.16 (0.10)</td>
<td>-0.17 (0.09)</td>
<td>0.01 (0.92)</td>
</tr>
<tr>
<td>Longevity</td>
<td>-</td>
<td>1.00</td>
<td>-0.01 (0.93)</td>
<td>0.62 (0.001)</td>
<td>-0.17 (0.09)</td>
</tr>
<tr>
<td>First-day fecundity</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>0.45 (0.001)</td>
<td>0.15 (0.13)</td>
</tr>
<tr>
<td>Total fecundity</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>-0.10 (0.33)</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* Values in parentheses indicate *P* values.
size showed a weak negative correlation with total fecundity (Table 3-1, -0.17, $P=0.09$) analysis of covariance (ANCOVA, results not shown) was carried, with size as a covariate to correct for the size effect. Because the results of the ANCOVA showed no size effect (the $F$ values were non-significant for all traits due to size), size was used as a variable rather than as a covariate in all further analyses.

A two-way unbalanced mixed model ANOVA with two parental females nested under each parental male was used to analyze the data for genotype x environment interaction. The two hosts (budworm and flour moth) were considered as a fixed factor and the full and half-sib families and interaction between the host, full- and half-sib families were considered as a random factor in the analysis. The main effect host type, estimated the extent of effect of hosts on the quality traits measured on all families together, and the main effect male and female (male) estimated genetic variation among full and half-sib families for both hosts (Via, 1984; Fry, 1992). The interaction effects (G x E) 'male x host' and 'female x host', were the most important part of the analysis, because they estimated genetic variation in phenotypic plasticity i.e., how the full and half-sib families varied in their phenotypic expression between the two hosts (Via, 1994).

All analyses were performed using ‘Type IV’ sums of squares from the General Linear Models procedure in SAS (SAS, 1989); as these were appropriate for an unbalanced design (Via, 1984). Type IV expected means squares, which took the unequal cell sizes into account, were also generated using SAS, to identify the appropriate error terms and to estimate variance components. The formula for the $F$ ratio was constructed to include the nested condition in testing the significance of each factor in the ANOVA model. The difference between means for each trait was tested for
significance using Cochran's $T$ test. Plasticity was measured as the difference in the overall mean values of a trait between the two hosts (Via, 1994).

Variance components in the above analysis provide information on how much of the observed variation in each trait was contributed by the host (environment) versus how much by the genotypes (full and half-sib families) (Falconer and Mackay, 1996). Because the sum of squares for each factor in an unbalanced ANOVA model is calculated by including the other factors in the model (Appendix 1), ANOVA does not fully reveal the relative contribution of the underlying causal components to phenotypic variance (Via, 1984). Thus, to augment the ANOVA, the observed components of variance were estimated by solving the system of simultaneous equations prescribed by the mean squares (Type IV) and their expectations (Appendix 1). The causal components were then estimated from the theoretical relationship between genetic variance and the observed variance among half and full-sib families for the haplo-diploid organisms (Appendix 2) (Liu, 1998).

**RESULTS**

Analysis of variance for quality traits such as size, longevity, first-day and total fecundity, and sex ratio of *T. minutum* indicates that the observed variation in all traits was due to the main effect of host (Table 3–2, host, $P < 0.05 - 0.001$). The main factors, male and female (male) were not significant for any character, suggesting that both full and half-sib families had no significant genetic variation among them for any of the character. Further, these non-significant values imply that all the experimental male and female parents were genetically identical to each other.
Table 3-2. Two-way mixed model nested ANOVA for arrhenotokous *Trichogramma minutum* reared on spruce budworm and Mediterranean flour moth eggs at 20°C.

<table>
<thead>
<tr>
<th>Source</th>
<th>df*</th>
<th>MS</th>
<th>F ratio</th>
<th>Size</th>
<th>Longevity</th>
<th>First day fecundity</th>
<th>Total fecundity</th>
<th>Sex ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MS</td>
<td>MS</td>
<td>MS</td>
<td>MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F value</td>
<td></td>
<td></td>
<td></td>
<td>MS</td>
<td>MS</td>
</tr>
<tr>
<td>Model</td>
<td>27</td>
<td>MS1</td>
<td>MS1/MS7</td>
<td>0.070</td>
<td>30.06**</td>
<td>32.80</td>
<td>6.04**</td>
<td>7599.50</td>
</tr>
<tr>
<td>M</td>
<td>6</td>
<td>MS2</td>
<td>MS2/MS3</td>
<td>0.003</td>
<td>0.41</td>
<td>19.01</td>
<td>1.53</td>
<td>5318.30</td>
</tr>
<tr>
<td>Fem (M)</td>
<td>7</td>
<td>MS3</td>
<td>MS3/MS6</td>
<td>0.008</td>
<td>1.35</td>
<td>12.45</td>
<td>0.83</td>
<td>2365.47</td>
</tr>
<tr>
<td>H</td>
<td>1</td>
<td>MS4</td>
<td>MS4/MS5</td>
<td>1.340</td>
<td>210.35**</td>
<td>343.72</td>
<td>22.90**</td>
<td>63986.11</td>
</tr>
<tr>
<td>M x H</td>
<td>6</td>
<td>MS5</td>
<td>MS5/MS6</td>
<td>0.006</td>
<td>1.10</td>
<td>15.01</td>
<td>1.00</td>
<td>2333.38</td>
</tr>
<tr>
<td>Fem (M) x H</td>
<td>7</td>
<td>MS6</td>
<td>MS6/MS7</td>
<td>0.006</td>
<td>2.33*</td>
<td>15.03</td>
<td>2.77**</td>
<td>1634.13</td>
</tr>
<tr>
<td>Error</td>
<td>164</td>
<td>MS7</td>
<td>-</td>
<td>0.003</td>
<td>-</td>
<td>5.43</td>
<td>415.80</td>
<td>1030.37</td>
</tr>
</tbody>
</table>

*df = degrees of freedom; MS = mean square; M = male parent; Fem (M) = female parent nested under a male parent; H = host; M x H = interaction between male parent and host; Fem (M) x H = interaction between female (M) and host. * indicate P < 0.05; ** indicate P<0.01.
The genotype x environment (host) interactions quantify genetic variation the full and half-sib families on different hosts. The interactions between male x host were not significant for all of the characters demonstrating that no genetic variation existed amongst the full sib families in their response to different rearing hosts (Table 3-2, male x host, NS). The interactions between the half-sib families x host for all the traits [female (male) x host, $P < 0.05 - 0.001$], however were significant except for total fecundity, suggesting that the phenotypic response of F$_1$ females varied depending on the type of host on which they were reared. The non-significant $F$ value for total fecundity suggests that there was no difference among the F$_1$ females in the number of progeny produced under a given host.

The variance components in Table 3-3 strongly support the ANOVA, in that the estimated values for observed and casual components of host ($V_{Host}$) were overwhelmingly larger for all traits compared to the values obtained for any variance component in the table. For example, 100% of the phenotypic variation in size was due to the rearing host ($V_{Host, Size}$). For other traits about 90 to 98% of the observed variation was caused by the difference in hosts. The additive ($V_{Male}$) and additive-interaction genetic variance [$V_{Male \times host}$] did not exceed 0.5% of the total phenotypic variance for any given trait. This explains the lack of significance for these two terms in the ANOVA. Similarly, the non-additive genetic variance was also negligible for all characters. Although the female (male) x host interactions were highly significant in the ANOVA, these terms contributed only up to a maximum of 4% of the total phenotypic variation in variance components estimation.
Table 3–3. Variance components for size, longevity, first-day fecundity, total fecundity, and sex ratio of arrhenotokous *Trichogramma minutum* reared on spruce budworm and Mediterranean flour moth eggs at 20°C. Observed components were obtained from the mean squares of the ANOVA (Appendix 1) and the casual components were estimated from a modified Falconer’s formula (Appendix 2).

<table>
<thead>
<tr>
<th>Components*</th>
<th>Size</th>
<th>Longevity</th>
<th>First-day fecundity</th>
<th>Total fecundity</th>
<th>Sex ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Casual</td>
<td>Observed</td>
<td>Casual</td>
<td>Observed</td>
</tr>
<tr>
<td>$V_{\text{Host}}$</td>
<td>1.30</td>
<td>1.30</td>
<td>(100.00)*b</td>
<td>329.47</td>
<td>329.74</td>
</tr>
<tr>
<td>$V_{\text{Male}}$</td>
<td>0.00</td>
<td>0.00</td>
<td>(0.00)</td>
<td>0.13</td>
<td>0.26</td>
</tr>
<tr>
<td>$V_{\text{Female (male)}}$</td>
<td>0.00</td>
<td>0.00</td>
<td>(0.00)</td>
<td>0.17</td>
<td>0.10</td>
</tr>
<tr>
<td>$V_{\text{Male x host}}$</td>
<td>0.00</td>
<td>0.00</td>
<td>(0.00)</td>
<td>0.37</td>
<td>0.74</td>
</tr>
<tr>
<td>$V_{\text{Male (male) x host}}$</td>
<td>-0.08</td>
<td>-0.15</td>
<td>(0.00)</td>
<td>1.81</td>
<td>3.24</td>
</tr>
<tr>
<td>$V_{\text{error}}$</td>
<td>0.002</td>
<td>0.002</td>
<td>(0.00)</td>
<td>5.43</td>
<td>5.43</td>
</tr>
<tr>
<td>$V_{\text{Total}}$</td>
<td>1.30</td>
<td></td>
<td></td>
<td>339.14</td>
<td></td>
</tr>
</tbody>
</table>

*a* $V_{\text{Host}}$ = variance due to host; $V_{\text{Male}}$ = variance due to male parent; $V_{\text{Female(male)}}$ = variance due to female parent; $V_{\text{Male x host}}$ = variance due to interaction between male parent and host; $V_{\text{Male(male) x host}}$ = variance due to interaction between female parent and host; $V_{\text{error}}$ = variance due to experimental error; $V_{\text{Total}}$ = total phenotypic variance (sum of all causal components).

*b* Numbers in parenthesis indicate the % contribution of each genetic component to the total genetic variation.
Reaction norm plots have been drawn for all the quality traits examined in this study to augment the genotype x environment interaction in the ANOVA. These plots graphically represent the response of each genotype (=family) to a change in the environment and had been drawn only for the half-sib families, because of their significant interactions [female (male) x host] in ANOVA. No significant variation in reaction norm plots was observed among the half-sib families for size, as all the families have higher mean size on budworm and relatively smaller mean size on flour moth (Fig. 3–2a). The reaction norms for longevity, first-day fecundity, total fecundity and sex ratio all showed differences in the mean values for the respective characters among various half-sib families (Fig. 3-2b & c and Fig. 3-3a & b). Although the general trend was towards a lower mean value for a given character on flour moth, a few families had significantly higher or lower values on flour moth compared to budworm, producing a crossing-over in the reaction norms. This could be the reason for the significant genotype x environment interaction in the ANOVA for these characters.

A clearer picture of how the quality and plasticity of *T. minutum* was affected as a result of host switching could be seen when the mean values of each quality trait were plotted for the entire population by host. Females reared on budworm were approximately 1.5 times larger than those from flour moth (Fig. 3-4a), lived 3 days longer when emerging from budworm (Fig. 3-4b), and their fecundity were 1.5 to 2 times higher than for those reared on flour moth (Fig. 3–5a & b). Although more female offspring were produced on both hosts, the % female offspring produced on budworm was significantly higher than that produced on flour moth (Fig. 3–5c). In other words, negative plasticity resulted when *T. minutum* was moved from budworm to flour moth.
Figure 3-2. Reaction norms for a) size, b) adult longevity, and c) sex ratio of *Trichogramma minutum* reared from two different host eggs. Each line connects the mean value from spruce budworm (SBW) and the Mediterranean flour moth (MFM) by a single half-sib family.
Figure 3–3. Reaction norms for a) first-day fecundity and b) total fecundity of *Trichogramma minutum* reared from two different host eggs. Each line connects the mean value from spruce budworm (SBW) and the Mediterranean flour moth (MFM) by a single half-sib family.
Figure 3-4. Realized mean values (± s. e.) for different traits of *Trichogramma minutum* reared on spruce budworm (SBW) and Mediterranean flour moth (MFM) eggs. a) size, b) adult longevity, and c) progeny sex ratio. Values plotted represent mean values for the entire population.
Figure 3-5. Realized mean values (± s. e.) for different traits of *Trichogramma minutum* reared on spruce budworm (SBW) and Mediterranean flour moth (MFM) eggs. a) first-day fecundity and b) total fecundity. Values plotted represent mean values for the entire population.
DISCUSSION

The study was conducted to examine whether *Trichogramma minutum* was plastic in response to different rearing hosts in terms of quality attributes such as parasitoid size, longevity, fecundity and progeny sex ratio. In addition, I was also interested in quantifying how much of the observed variation in each of the traits was caused by the rearing host and how much by genetic differences among the parasitoids. Switching the wasps from budworm to flour moth resulted in plastic changes in all of the quality traits examined. A significant host effect was observed indicated that host eggs were the only factor that influenced the observed phenotypic changes in these characters. These results are similar to those reported by Bourchier *et al.* (1994) for fecundity and by Corrigan and Laing (1994) for longevity, fecundity, sex ratio in *T. minutum*, where a reduction in all these characters were observed when the parasitoids were switched from budworm to flour moth.

Non-significant genotype x environment interaction between male x host suggested a lack of additive genetic variance among the full-sib families for plasticity. This was further substantiated by the variance components, which showed <0.5 % of additive genetic variation among the full-sibs. Although the interactions were highly significant between female (male) x host, the amount of non-additive genetic variation contributed by the half-sib families was about 4 % of the total phenotypic variation. This low non-additive genetic variance as illustrated by the plots of norms of reaction, showed that only a few half-sib families changed their ranking across the two hosts for a given trait.
A key parameter that determines the rate and direction of evolution of mean plasticity is the additive genetic variation for phenotypic plasticity expressed by a population in a heterogeneous environment (Via and Lande, 1985; Via, 1987; De Jong, 1990; Gomulkiewicz and Kirkpatrick, 1992; Scheiner, 1993). Lack of genetic variation means there is a limited potential for evolution of plastic responses. There are several possible explanations for the very low genetic variation seen in my study. First, the parasitoids used came from a single population, which was maintained in the laboratory for more than 60 generations on budworm. Second, it may be that my sample of 50 wasps (10 males and 40 females), which started the parental generation allowed for only a limited amount of genetic variation to be present. Finally, it could be that my the experimental population had already evolved to adapt to budworm due to the long period of rearing and the natural selection and this would have depleted all the additive genetic variation available in the population. This final point was supported by the reduction in mean values for each trait when Trichogramma was switched from budworm to flour moth (Figs. 3–2a, b, c and 3–3a &b).

The overall higher mean values for all traits obtained on budworm further indicate that the parasitoids had adapted to budworm as a rearing host. Also, with budworm being their natural host, it may be that they have an innate affinity for budworm and because flour moth was a new host, full potential during the first few generations may not be realized. Mal-adaptations can occur in populations when they are expanding their range in new host species (Via, 1987). Natural selection may be stronger for Trichogramma on flour moth than on budworm because the mean values obtained on this host during the first few generations was probably lower than the optimal that could be realized on flour
moth. It may take several generations for the parasitoids to adapt to the new host and evolution may be rapid for the quality traits on flour moth. Kaiser et al. (1989) have shown that long-term rearing of *T. maidis* on flour moth can enhance the affinity for this un-natural host; % parasitism was higher on this host after the parasitoids had had previous ovipositional experience on flour moth. So, long-term rearing of *T. minutum* on flour moth, measuring and comparing the trait mean values from each generation with those obtained in the F₁ generation, may provide useful information on whether the mean values obtained are, in fact the optimal ones for flour moth, or whether *Trichogramma* has adapted to flour moth.

It should be noted that the host characteristics of flour moth eggs should also be considered when assessing the inferiority of flour moth to budworm as a host. For example, the size of flour moth eggs is small compared to budworm and only a single parasitoid can develop from them. Because the size of the emerging wasp depends on the size of the host eggs, and there is a positive correlation between wasp size and other fitness traits (smaller the size the lesser the fecundity, lower the sex ratio etc.), it may not be possible for the mean values from flour moth to be any higher than those in the present study, simply due to limitations in size. In other words, the plastic response to flour moth although considered mal-adaptive when compared to that on budworm, may be optimal.
In conclusion, my work shows that there is plasticity in quality traits of *T. minutum* in response to host switching. The hosts used in my study caused 90 to 100% of the observed phenotypic variation. This plastic response on flour moth was negative, because the mean values obtained on flour moth were significantly lower than those observed on budworm although this may be associated with host size rather than other attributes. My findings also demonstrate that additive genetic variation for plasticity in response to host switching is negligible and that the potential for evolution of plasticity is relatively limited in this population.
Chapter Four: Phenotypic plasticity of quality characters for Trichogramma minutum Riley in response to rearing temperature

INTRODUCTION

Parasitoids belonging to the genus Trichogramma have been used worldwide as candidates for the biological control of various agricultural and forest insect pests. *T. minutum* has been developed as an alternative to the chemical control of eastern spruce budworm, *C. fumiferana* in forest environments (Smith *et al.*, 1990). Large-scale rearing systems have been developed to mass rear *Trichogramma* for inundative releases in North America, Europe, and Russia. However, the quality of *Trichogramma* has been reported to vary depending on the environmental conditions used for mass rearing (Bigler, 1991; Smith, 1996).

The influence of host characteristics on parasitoid quality has received considerable attention. In addition to the rearing host species, researchers have studied the influence of temperature on parasitoid quality attributes such as longevity, fecundity, emergence, parasitoid sex ratio and host acceptance (Pak and Oatman, 1982; Calvin *et al.*, 1986; Cabello and Vargas, 1988; Forsse *et al.*, 1992; Pavlik, 1992; Bourchier and Smith, 1996). Unfortunately, these studies have focussed mainly on what the changes in quality attributes mean for the efficiency of parasitoids in biological control rather than exploring the basis of this variation and how it affects the success and survival of parasitoids in the field.
Environmentally-induced phenotypic variation is known as phenotypic plasticity (Scheiner, 1993; Via, 1994) and estimation of plasticity can provide insights into the basis of phenotypic variation, as well as the type of environmental influence on parasitoid quality variation. Because rearing temperature has been reported to cause variation in quality attributes, I have estimated the extent of plasticity of quality characters at different temperatures in this chapter. Most *Trichogramma* used in inundative release programs are arrhenotokous (virgin females produce male offspring). Wang (1994) discovered a thelytokous *T. minutum* (virgin females almost always produce female offspring) and compared the arrhenotokous and thelytokous forms to study their biological and physiological attributes of parasitism. In this chapter, I have compared the phenotypic plasticity of these two reproductive forms to detect whether differences in quality attributes of arrhenotokous and thelytokous *T. minutum* were due to difference in their plasticity. Because the thelytokous population consists of genetically identical individuals, it provides a model system to quantify the exact amount of environmentally-induced phenotypic variation. Similar to the host switching experiment, I have estimated variance components to partition the observed variations into variance due to rearing temperature and variance due to genetic difference among the parasitoids. The results are discussed in terms of their implications for mass rearing of *Trichogramma*.

**MATERIALS AND METHODS**

**Parasitoid colony**

The arrhenotokous population of *T. minutum* from the host switching experiment was used in the present experiment. After completion of the host switching experiment,
the colonies were maintained on flour moth eggs for another ca. 10 generations at 20°C and 16=8 light-dark conditions. Once the parasitoids had multiplied sufficiently (>5,000 individuals) on flour moth, the experiment was initiated.

The thelytokous population used in the experiment originated from parasitized eggs of the spruce budmoth, *Zeiraphera canadensis* Mutuura & Freeman (Lepidoptera: Olethreutidae), collected in a young white spruce plantation in Restigouche, northern New Brunswick (47.7°W, 67°N) during 1997. The thelytokous population was maintained on flour moth eggs under the same conditions as the arrhenotokous population from the time of its collection. For the experiment, one iso-female line was initiated by allowing a single female to oviposit on flour moth eggs for 24 h. The parasitized eggs were incubated under the same conditions as the original population and the progeny was allowed to multiply for several generations without overlapping between generations. The population size was maintained at around 5,000 individuals in each generation for both the arrhenotokous and the thelytokous populations. There was no apparent cross movement of individuals between the two populations.

**Experimental procedure**

For the arrhenotokous population, a sib-family design was used to test the same genotype across different temperatures and the crossing procedure was similar to that of the host switching experiment. A total of 60 virgin females and 15 virgin males from the same cohort were mated at a ratio of 1 male: 4 females for two hours to initiate half- and full-sibs families in the parental generation. At the end of two hours, each mated female was placed in a small glass vial (3.5 x 1.5cm) and given flour moth eggs *ad libitum* for 24
h in a no-choice trial. After 24 h, the egg card was cut into three equal pieces and one piece was placed at 25, 20 and 15°C respectively. This procedure was repeated once to make sure that there were at least 5 to 10 parasitized eggs at each temperature. A drop of 50 % honey solution was streaked along the side of the vials as a food source during the trial. Only those parental females which had at least a few parasitized eggs at all three temperatures were included for further observation; this reduced the number of full-sib families from 60 to 28 (2 females/male).

The parasitized eggs were incubated at 25, 20 and 15°C and 16=8 light=dark conditions until the F1 generation emerged. The remaining procedures for the F1 generation were similar to the host switching experiment. Data on longevity were recorded from F1 females and the first-day fecundity (no. of progeny produced on the first day of adult life), total fecundity (total no. of progeny), sex ratio (% females) and % emergence of the progeny were recorded from the F2 generation. The % emergence of the progeny was calculated by dividing the total number of progeny emerged by the total number parasitized (black) eggs.

For the thelytokous population, the same procedure was repeated except that there was no mating. In total, 50 iso-females were used in the parental generation, but only 23 females were found to have parasitized eggs at all three temperatures. Longevity, first-day fecundity, total fecundity and % emergence were the parameters recorded for this population.
Statistical Analyses

All analyses were similar to that in the host switching experiment (Chapter 3). The sample size in the F1 generation varied from 3 to 15 per parental female for both populations. For the thelytokous population, the iso-females and the interaction between the ‘iso-females x temperature’ were both considered as the random factors. Variance components could not be estimated for the thelytokous population because the genotype was assumed to be same for all females and all observed variation was assumed to be caused by the rearing temperatures.

RESULTS

Arrhenotokous population

All of the variation in quality traits for the arrhenotokous population was contributed by the main factor ‘temperature’, except for the progeny sex ratio (Table 4–1, sex ratio, $F = 2.43$, NS). This significant temperature effect implies that the three temperatures chosen for the study were experienced as three different environments by $T. minutum$. Significant genetic variation could be detected among the full-sib families only for total fecundity (Table 4–1, male, $F = 4.54$, $P < 0.001$). All the half-sib families were identical in their response to the experimental temperatures as no significant difference was found for any of the traits [Table 4–1, female (male), NS].
Table 4–1. Two-way mixed model nested ANOVA for arrhenotokous *Trichogramma minutum* reared on Mediterranean flour moth eggs at 15, 20 and 25°C.

<table>
<thead>
<tr>
<th>Source</th>
<th>df $^a$</th>
<th>MS</th>
<th>$F$ ratio</th>
<th>Longevity</th>
<th></th>
<th>First-day fecundity</th>
<th></th>
<th>Total fecundity</th>
<th></th>
<th>Emergence</th>
<th></th>
<th>Sex ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MS</td>
<td>$F$ value</td>
<td>MS</td>
<td>$F$ value</td>
<td>MS</td>
<td>$F$ value</td>
<td>MS</td>
<td>$F$ value</td>
<td>MS</td>
</tr>
<tr>
<td>Model</td>
<td>83</td>
<td>MS1</td>
<td>MS1/MS7</td>
<td>151.82</td>
<td>13.57**</td>
<td>1314.67</td>
<td>8.92**</td>
<td>6265.52</td>
<td>12.40**</td>
<td>659.12</td>
<td>5.91**</td>
<td>781.73</td>
</tr>
<tr>
<td>M</td>
<td>13</td>
<td>MS2</td>
<td>MS2/MS3</td>
<td>65.32</td>
<td>1.81</td>
<td>488.72</td>
<td>1.61</td>
<td>4801.34</td>
<td>4.54**</td>
<td>527.56</td>
<td>2.28</td>
<td>860.27</td>
</tr>
<tr>
<td>Fem (M)</td>
<td>14</td>
<td>MS3</td>
<td>MS3/MS6</td>
<td>36.04</td>
<td>1.00</td>
<td>302.99</td>
<td>0.69</td>
<td>1058.07</td>
<td>0.94</td>
<td>231.84</td>
<td>1.09</td>
<td>518.25</td>
</tr>
<tr>
<td>T</td>
<td>2</td>
<td>MS4</td>
<td>MS4/MS5</td>
<td>2958.46</td>
<td>37.02**</td>
<td>30733.11</td>
<td>44.46**</td>
<td>119057.36</td>
<td>54.45**</td>
<td>9166.46</td>
<td>26.98**</td>
<td>2021.66</td>
</tr>
<tr>
<td>M x T</td>
<td>26</td>
<td>MS5</td>
<td>MS5/MS6</td>
<td>79.92</td>
<td>2.23*</td>
<td>691.23</td>
<td>1.57</td>
<td>2186.61</td>
<td>1.94*</td>
<td>339.72</td>
<td>1.60</td>
<td>830.50</td>
</tr>
<tr>
<td>Fem (M) x T</td>
<td>28</td>
<td>MS6</td>
<td>MS6/MS7</td>
<td>35.87</td>
<td>3.21**</td>
<td>439.85</td>
<td>2.98**</td>
<td>1129.21</td>
<td>2.23**</td>
<td>212.82</td>
<td>1.92**</td>
<td>553.33</td>
</tr>
<tr>
<td>Error</td>
<td>367</td>
<td>MS7</td>
<td></td>
<td>11.19</td>
<td></td>
<td>147.40</td>
<td></td>
<td>505.29</td>
<td></td>
<td>111.57</td>
<td></td>
<td>270.23</td>
</tr>
</tbody>
</table>

* $df = $ degrees of freedom; MS = mean square; M = male parent; Fem (M) = female parent nested under a male parent; T = temperature; M x T = interaction between male parent and temperature; Fem (M) x T = interaction between female (M) and temperature. * indicate $P < 0.05$; ** indicate $P < 0.001$. 

$^a$
Genotype x environment interaction (genetic variation for plasticity) was investigated by comparing 'male x temperature' and 'female (male) x temperature' interactions (Table 4-1). The 'male x temperature' interaction was significant for longevity ($F = 2.23, P < 0.05$) and total fecundity ($F = 1.94, P < 0.05$) suggesting that there was additive interaction variance for these two characters and that the full-sib families differed in their average response to rearing temperature for these two traits. The 'female (male) x temperature' was highly significant ($P < 0.001$) for all quality traits, implying that the half-sib families were also significantly different in their phenotypic response to the experimental temperatures.

When the variance components were examined, the results obtained from the ANOVA were supported further; 74 to 99% of the observed variation in quality traits was contributed by the rearing temperature (Table 4-2, $V_{Temperature}$), while <0.5% of the total genetic variation in total fecundity, emergence and sex ratio was due to additive genetic variance (Table 4-2, $V_{Male}$). Although the results of the ANOVA were non-significant for emergence and sex ratio.
Table 4–2. Variance components for longevity, first-day fecundity, total fecundity, emergence rate and sex ratio of arrhenotokous *Trichogramma minutum* reared on Mediterranean flour moth eggs at 15, 20 and 25°C. Observed components were obtained from the mean squares of the ANOVA (Appendix 1) and the casual components were estimated from modified Falconer's formula (Appendix 2).

<table>
<thead>
<tr>
<th>Components*</th>
<th>Longevity</th>
<th>First day fecundity</th>
<th>Total fecundity</th>
<th>Emergence</th>
<th>Sex ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Casual</td>
<td>Observed</td>
<td>Casual</td>
<td>Observed</td>
</tr>
<tr>
<td>V Temperature</td>
<td>2682.76 (98.98)</td>
<td>30075.30 (98.98)</td>
<td>116974.18 (99.03)</td>
<td>8840.74 (97.93)</td>
<td>1225.54 (74.00)</td>
</tr>
<tr>
<td>V Male</td>
<td>-0.50 (0.00)</td>
<td>-2.00 (0.00)</td>
<td>100.94 (0.17)</td>
<td>6.41 (0.14)</td>
<td>2.71 (0.33)</td>
</tr>
<tr>
<td>V Female(male)</td>
<td>0.08 (0.02)</td>
<td>-9.41 (0.00)</td>
<td>-3.72 (0.00)</td>
<td>1.66 (0.00)</td>
<td>-1.90 (0.00)</td>
</tr>
<tr>
<td>V Male x temperature</td>
<td>4.77 (0.35)</td>
<td>27.30 (0.18)</td>
<td>114.41 (0.20)</td>
<td>13.75 (0.30)</td>
<td>30.07 (0.33)</td>
</tr>
<tr>
<td>V Fem(male) x temperature</td>
<td>5.30 (0.22)</td>
<td>62.80 (0.32)</td>
<td>133.90 (0.13)</td>
<td>21.73 (0.33)</td>
<td>60.76 (5.52)</td>
</tr>
<tr>
<td>V error</td>
<td>11.20 (0.43)</td>
<td>147.40 (0.51)</td>
<td>505.30 (0.47)</td>
<td>115.57 (1.30)</td>
<td>270.23 (16.51)</td>
</tr>
<tr>
<td>V Total</td>
<td>2710.31 (98.98)</td>
<td>30383.93 (98.98)</td>
<td>118118.05 (99.03)</td>
<td>9027.90 (98.98)</td>
<td>1656.03 (74.00)</td>
</tr>
</tbody>
</table>

* V Temperature = variance due to temperature; V Male = variance due to male parent; V Female(male) = variance due to female parent; V Male x temperature = variance due to interaction between male parent and temperature; V Fem(male) x temperature = variance due to interaction between female parent and temperature; V error = variance due to experimental error; V Total = total phenotypic variance (sum of all casual components).

b Numbers in parenthesis indicate the % contribution of each genetic component to the total genetic variation.
The variation due to non-additive genetic variance was negligible as the values obtained for both the observed and causal components were either negative or zero for all characters. The variance components also indicated that 0.2 to 4 % of the total phenotypic variation observed in the traits was due to the additive interactions (Table 4–2, \(V_{\text{Male} \times \text{temperature}}\)) even though the 'male x temperature' interactions were significant only for longevity and total fecundity. The non-additive interactions (\(V_{\text{Fem (male) x temperature}}\)) accounted for 0.2 to 6 % of the variation although these interactions were highly significant for all traits in the ANOVA.

The population mean values can be used to show the mean reaction norms for different families across the temperatures because of the large number of full and half-sib families. The shape of the reaction norm for longevity was linear with longevity decreasing as the temperature increased (Fig. 4–1a). The responses for first-day fecundity and total fecundity were opposite to longevity, where the reaction norms were convex with the maximum value for these two characters expressed at 20°C and significantly lower values expressed at 15°C. The number of progeny produced on the first day of emergence was the same at 20 and 25°C (Fig. 4–2a & b). The shape of the reaction norms for emergence and sex ratio was similar to fecundity, with emergence being non-significant at 20 and 25°C and sex ratio being non-significant at 15 and 25°C (Fig. 4–1b & c). Longevity, first-day and total fecundity were highly plastic based on the large variation observed among the mean values at any two temperatures, while the mean values for emergence and sex ratio were more similar and hence less plastic.
Figure 4–1. Reaction norms (mean ± s. e.) for a) adult longevity; b) emergence, and c) progeny sex ratio of arrhenotokous *Trichogramma minutum* reared on Mediterranean flour moth eggs at 15, 20 and 25°C.
Figure 4–2. Reaction norms (mean ± s. e.) for a) first-day fecundity and b) total fecundity of arrhenotokous *Trichogramma minutum* reared on Mediterranean flour moth eggs at 15, 20 and 25°C.
The reaction norm plots suggest that 20°C was the best rearing temperature for *T. minutum*, as almost all quality traits measured have higher values at this temperature than at the other two temperatures. Temperatures above or below 20°C appear to decrease the quality characters due to negative plasticity or mal-adaptation.

**Thelytokous population**

The thelytokous females differed significantly in their response to the experimental temperatures except for first-day fecundity (Table 4–3, female, \(P<0.001\)). Similarly, the main factor 'temperature' also had a significant effect on all the females for all the observed traits. Genotype x environment interaction between ‘female x temperature’ showed that the iso-females differed significantly in their phenotypic expression according to the rearing temperature.

Reaction norm plots were drawn using the mean response of the entire population to show the response of individual families across temperatures. The shapes of the reaction norms for longevity, first-day fecundity, and total fecundity were similar to those of the arrhenotokous population (Figs. 4-3a and 4–4 a & b) although first-day fecundity and total fecundity were non–significant at 20 and 25°C. The reaction norm plot for emergence was flat with emergence being > 85 % at all three temperatures (Fig. 4–3b).
Table 4-3. Two-way mixed model ANOVA for thelytokous *Trichogramma minutum* reared on Mediterranean flour moth eggs at 15, 20 and 25°C.

<table>
<thead>
<tr>
<th>Source</th>
<th>$df^a$</th>
<th>Longevity</th>
<th>First-day fecundity</th>
<th>Total fecundity</th>
<th>Emergence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MS</td>
<td>$F$ value</td>
<td>MS</td>
<td>$F$ value</td>
</tr>
<tr>
<td>Model</td>
<td>74</td>
<td>18.78</td>
<td>6.39**</td>
<td>88.90</td>
<td>5.20**</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td>6.42</td>
<td>2.19**</td>
<td>15.50</td>
<td>0.91</td>
</tr>
<tr>
<td>Temperature</td>
<td>2</td>
<td>321.57</td>
<td>109.44**</td>
<td>2002.20</td>
<td>117.06**</td>
</tr>
<tr>
<td>Female x Temperature</td>
<td>48</td>
<td>3.37</td>
<td>3.37**</td>
<td>25.54</td>
<td>1.49*</td>
</tr>
<tr>
<td>Error</td>
<td>326</td>
<td>2.93</td>
<td>17.10</td>
<td>36.30</td>
<td></td>
</tr>
</tbody>
</table>

*df = degrees of freedom; MS = mean square; * indicate $P < 0.05$; ** indicate $P < 0.001$. 
Figure 4-3. Reaction norms (mean ± s. e.) for a) adult longevity and b) emergence of thelytokous *Trichogramma minutum* reared on Mediterranean flour moth eggs at 15, 20 and 25°C.
Figure 4-4. Reaction norms (mean ± s. e.) for a) first-day fecundity and b) total fecundity of thelytokous *Trichogramma minutum* reared on Mediterranean flour moth eggs at 15, 20 and 25°C.
Although the different temperatures have induced plasticity for all characters, the extent of the plasticity was less for the thelytokous population compared to that of the arrhenotokous population between any two temperatures due to a smaller difference in the mean values. These reaction norm plots imply that rearing the thelytokous population at 20 or 25°C did not change any of the quality traits.

**Comparison between arrhenotokous and thelytokous population**

There was a significant difference in longevity, first-day fecundity, total fecundity and emergence between the arrhenotokous and thelytokous populations (Table 4–4, population, $P < 0.001$) due to the difference in the population. Rearing temperature also had similar influence on these same characters. The significant ‘population x temperature’ interaction effect for all quality traits indicated that the phenotypic responses of both populations differed according to the rearing temperature.

The mean longevity for the arrhenotokous population was approximately twice as high as that for the thelytokous population (Fig. 4–5a), while mean first-day fecundity was 3-fold higher (Fig. 4–6a), mean total fecundity was 4 times higher at 15 and 25°C, and 5 times higher at 20°C than that for the thelytokous population (Fig. 4–6b). An important point to note here was that the increase in longevity might have resulted from a trade-off with first-day and total fecundity at 15°C for both populations. The emergence was slightly higher for the thelytokous *T. minutum* at 15°C than the arrhenotokous population (Fig. 4–5 b) and there was no difference between the two populations at 20 and 25°C.
Table 4–4. Two-way mixed model ANOVA for arrhenotokous and thelytokous *Trichogramma minutum* reared on Mediterranean flour moth eggs at 15, 20 and 25°C.

<table>
<thead>
<tr>
<th>Source</th>
<th>$df^a$</th>
<th>Longevity</th>
<th>First-day fecundity</th>
<th>Total fecundity</th>
<th>Emergence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MS</td>
<td>$F$ value</td>
<td>MS</td>
<td>$F$ value</td>
</tr>
<tr>
<td>Model</td>
<td>5</td>
<td>2752.82</td>
<td>227.08**</td>
<td>37456.04</td>
<td>288.47**</td>
</tr>
<tr>
<td>P</td>
<td>1</td>
<td>3707.41</td>
<td>305.83**</td>
<td>111982.16</td>
<td>862.37**</td>
</tr>
<tr>
<td>T</td>
<td>2</td>
<td>3361.05</td>
<td>277.26**</td>
<td>24331.36</td>
<td>187.38**</td>
</tr>
<tr>
<td>P x T</td>
<td>2</td>
<td>822.45</td>
<td>67.84**</td>
<td>6616.76</td>
<td>50.96**</td>
</tr>
<tr>
<td>Error</td>
<td>772</td>
<td>12.12</td>
<td>129.85</td>
<td>502.81</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ df = degrees of freedom; MS = mean square; P = population; T = temperature; P x T = interaction between population and temperature. ** indicate $P < 0.001$. 
Figure 4-5. Realized mean values (± s. e.) for a) adult longevity and b) emergence of arrhenotokous and thelytokous *Trichogramma minutum* reared on Mediterranean flour moth eggs at 15, 20 and 25°C. Values plotted represent the mean values for entire population.
Figure 4–6. Realized mean values (± s. e.) for a) first-day fecundity and b) total fecundity of arrhenotokous and thelytokous *Trichogramma minutum* reared on Mediterranean flour moth eggs at 15, 20 and 25°C. Values plotted represent the mean values for entire population.
DISCUSSION

Results from my study clearly indicate that the observed variation in all the quality traits was due to rearing temperatures and the interaction between temperatures x genotypes. This suggests that the variation in quality attributes resulted from phenotypic plasticity in the arrhenotokous and thelytokous populations and confirms the earlier findings about phenotypic plasticity for lifetime fecundity of arrhenotokous *T. minutum* (Liu, 1998). However, my findings about the plasticity of adult longevity and sex ratio were different from that of Liu (1998), which I will discuss later. Variance components for arrhenotokous *T. minutum* support the findings of significant phenotypic plasticity from the ANOVA, with 74 to 99 % of the total phenotypic variation accounted for by the rearing temperature. Total genetic variance (additive and non-additive) constituted <10 % of the total phenotypic variation for the different characters, of which the additive genetic variance accounted for <4 %. In general, the negative values obtained for the different genetic components suggest that there is low genetic variation in the experimental populations. Negative values can also result from the way the variance components are estimated (Via, 1984). Negative values for non-additive variance ($V_{Female \ (male) \ x \ temperature}$) may result because female parents are nested under male parents and this may mask the effect of female parents contributing to the total observed variation. Therefore, some of the variance in the full-sib families may be due to phenotypic differences among the female parents rather than due to the additive genetic variance contributed to by the male parents alone.

Low temperature appears to prolong adult longevity in arrhenotokous and thelytokous populations and any rise in temperature above 15 ºC will reduce the number
of days the adults live. Similar observations have been made for many other Trichogramma spp (Para et al., 1990; Garcia and Tavares, 1994; Wang and Smith, 1996; McDougall and Mill, 1997), the milkweed bug, O. faciatus (Groeters and Dingle, 1988) and D. melanogaster (Nunny and Cheung, 1997). However, my finding disagrees with that of Liu (1998) where longevity of T. minutum was highest at 20°C. It is possible that the difference lies in the fact that my study looked at one strain, while Liu (1998) examined 13 strains.

Norms of reaction for fecundity (first-day and total) observed in the present study show that it was highest at 20°C, confirming the fact that females raised at low or high temperatures will typically be less fecund than those raised at intermediate temperatures (Delpuech et al., 1995; Liu, 1998). My results contradict those observed by Antolin (1992) for M. raptor and Huey et al. (1995) for D. melanogaster, where there was no genotype x environment interaction between fecundity and rearing temperature; both of these studies found fecundity to increase with increasing temperature. As suggested by Liu (1998), the difference between my study and those of Antolin (1992) and Huey et al. (1995) may be due to either the difference in the species studied or how fecundity was measured. In M. raptor, fecundity was measured only for the first two days of adult emergence and in D. melanogaster only for first five days. In my study, fecundity was measured from the first day of emergence until the females died. In T. minutum, Liu (1998) showed that G x E interaction occurs at day 1, 2 and 4 to 6 days of emergence. Thus, the significant difference for observed fecundity in my study and Liu (1998) can be attributed to differences in G x E. In the other two studies, G x E did not seem to arise until later in life for M. raptor and D. melanogaster, because fecundity measured on the
first five days showed no difference among the test temperatures. It is also possible that the measurement of lifetime fecundity might have produced differences in fecundity at the different temperatures.

Low temperature seemed to affect emergence in the arrhenotokous population, whereas emergence of thelytokous *T. minutum* was unaffected by the different temperatures. Sex ratio was reduced at low and high temperatures; this contradicting the results of both Antolin (1992) and Liu (1998), who found a linear relationship between sex ratio and temperature. This discrepancy could be due to the fact that either only a single population was examined in my present study or because of the phenotypic correlation between fecundity and sex ratio (Antolin, 1992). In *M. raptor*, Antolin (1992) found a positive correlation between fecundity and sex ratio, and strains with higher fecundity produced a more female-biased sex ratio. The same reasoning could apply to my study, where fecundity was lower at 15 and 25 °C than at 20°C, as was also sex ratio at these two temperatures.

The arrhenotokous and thelytokous populations differed significantly in all traits as reported by Wang and Smith (1996). Except for emergence, for any given trait there was a 2- to 5-fold difference in plasticity between the two populations. Higher plasticity in the arrhenotokous population could result from its ecological range. Arrhenotokous *T. minutum* have a wide North American distribution in arboreal habitats (Pinto et al., 1992) and undoubtedly experience a wide range of temperature conditions. A higher level of plasticity might be expected to evolve in such populations, which encounter more variable thermal environments (Partridge et al., 1994; Worthen, 1996). On the other hand, thelytoky is rare in *T. minutum* and the thelytokous population in my study appears
to have a restricted distribution and to be closely synchronized with its primary host, *Z. canadensis* (Wang, 1994). Hence it may be less plastic than the arrhenotokous population.

In general, hymenopterans have been reported to be less heterozygous (Avery, 1984) than other insects. This was supported by the low genetic variation found among the full and half-sib families in my study. Based on this, it might be expected that *T. minutum* will have low environmental tolerance and narrow ecological distribution. However, from my study, it is clear that plasticity could be an important factor in the geographical extension of *Trichogramma*. However, further evolution of plasticity might be limited in the population due to low additive genetic variance. Low plasticity in the thelytokous population suggests that sub-populations of *Trichogramma* have evolved to adapt to a variety of hosts and thermal conditions.

In summary, rearing *Trichogramma* minutum at 15, 20 and 25°C demonstrated plasticity in the quality traits of both arrhenotokous and thelytokous populations; 75 to 99 % of the observed variation is a result of plasticity. Arrhenotokous *Trichogramma* had equal or higher mean values for all characters at any given temperature as thelytokous ones. Longevity, first-day fecundity and total fecundity were highly plastic, while emergence and sex ratio were less plastic. Thelytokous *Trichogramma* appear to be more specialized due to their lower performance and plasticity. Because my study failed to detect high genetic variation in the arrhenotokous population, it is suggested that this population has a limited capacity to evolve plastic changes with respect to rearing temperature.
Chapter Five: Summary

Plasticity due to host switching

Phenotypic plasticity of *Trichogramma minutum* was examined to determine how and why quality attributes vary depending on the environmental conditions used for rearing. My thesis is the first to compare the arrhenotokous and thelytokous reproductive forms of *T. minutum* and address whether the difference in quality between the two forms is due to plasticity. Plasticity due to host switching was measured for life history traits such as adult size, longevity, fecundity and progeny sex ratio. My experiments showed that switching the rearing host of the parasitoids from spruce budworm eggs to Mediterranean flour moth eggs resulted in negative reaction norms for all observed characters, e.g. mean values obtained on budworm were higher compared to those observed on flour moth.

Parasitoid size, longevity, first-day and total fecundity were highly plastic, whereas the sex ratio of the progeny was less plastic. Variance components further showed that the rearing host caused 74 to 100 % of the observed phenotypic variation in each trait. Genetic differences among the parasitoids accounted for only up to 10 % of the variation in parasitoid quality. Additive genetic variance was estimated as <0.5 % of the total phenotypic variation and was attributed to the fact that only a single population examined with a small number of individuals starting the parental generation.
Plasticity due to rearing temperature

Arrhenotokous and thelytokous *T. minutum* were reared at 15, 20 and 25°C to estimate plasticity in quality traits like adult longevity, first-day fecundity, total fecundity, and emergence and progeny sex ratio. Arrhenotokous *Trichogramma* had a negative linear reaction norm for longevity, with the number of days lived decreasing as the temperature increased from 15 to 25°C. The remaining traits had convex reaction norms, with higher values expressed at 20°C and lower values at 15 and 25°C. Longevity, first-day fecundity and total fecundity were highly plastic, whereas emergence and sex ratio were less plastic in arrhenotokous population. Variance components showed that the rearing temperature caused 90 to 98 % of the total phenotypic variation with <4 % of the variation due to genetic variation among the parasitoids.

The reaction norms for longevity, first-day fecundity and total fecundity of the thelytokous population were similar to those of the arrhenotokous population. However, the reaction norm for emergence was flat across the temperatures, with a slight difference between 15°C and rest of the temperatures for the thelytokous population. Even though the different temperatures resulted in plasticity for the quality characters, the extent of plasticity was always less for the thelytokous *Trichogramma* than for the arrhenotokous population.

Arrhenotokous *Trichogramma* appear to have higher plasticity for longevity, first-day and total fecundity than thelytokous populations at any given temperature. Emergence was similar and >75 % except at 15°C for both populations. Based on my experimental results, 20°C would be the ideal rearing temperature for both arrhenotokous
and thelytokous *T. minutum* as all the quality traits observed were higher or equal to the other two temperatures except for longevity.

**Implications**

**Ecological and evolutionary significance**

Host-induced plasticity may have ecological significance if it enhances individual performance in different environments or increases species niche breadth. My results indicate that host-induced plasticity decreases individual fitness for those parasitoids reared on flour moth and that *Trichogramma* encountering hosts of inferior quality to budworm may be able to adapt to the new host, but that their parasitism will never be higher than on budworm due to reduced fitness. On the other hand, this new host may serve to increase the parasitoids’ niche and help it survive when the availability of budworm is limited.

Arrhenotkous *T. minutum* appear to have evolved higher plasticity than thelytokous populations to withstand the fluctuating temperature conditions found across their ecological range. Lower plasticity in the thelytokous *Trichogramma* suggests that this reproductive form has evolved specifically to attack its primary host and that it is adapted to relatively cool environmental conditions prevailing in northern New Brunswick. Because this population has specialized on spruce budmoth, further evolution may be constrained unless environmental conditions force them to find another host. The low genetic variance found both in the host switching and temperature experiments imply that further evolution may be constrained and mal-adaptations can occur if the arrhenotokous population is exposed to host species of inferior quality and
temperatures above 20°C thus driving the population away from the optimum phenotypic value obtained on budworm.

Implications for mass rearing

From an applied perspective, plasticity in quality traits is advantageous because parasitoid quality can be improved by simply changing the rearing conditions. My study shows that rearing *T. minutum* at 20°C on budworm would produce better quality parasitoids than those reared on flour moth. Long-term rearing under constant conditions has been suggested to reduce the field efficiency of *Trichogramma* however, my study shows that parasitoids can adapt to field conditions due to their plasticity and long term rearing may not drastically change their quality. In addition, plasticity of a population should be considered as another criterion when selecting stains for mass rearing. Thelytokous populations have been suggested as suitable candidates for biological control due to their higher female production and colonization rates; however, these attributes alone may not be sufficient to make such a recommendation. Comparisons between arrhenotkous and thelytokous *T. minutum* clearly suggest that the arrhenotkous population would be a better candidate due to its high level of plasticity and should be considered as the best potential candidate for mass rearing and inundative release.
REFERENCES


APPENDICES

Appendix 1

Equations used to calculate the observed components in variance components estimation for the host switching experiment.

\[ V_{\text{male}} = \text{Variance (error)} + 5.2443 \text{ variance } [\text{fem (male) x host}] + 10.489 \text{ variance (male x host)} + 10.489 \text{ variance } [\text{fem (male)}] + 20.977 \text{ variance (male)} \]

\[ V_{\text{fem (male)}} = \text{Variance (error)} + 5.3158 \text{ variance } [\text{fem (male) x host}] + 10.632 \text{ variance } [\text{fem (male)}]. \]

\[ V_{\text{host}} = \text{Variance (error)} + 4.8306 \text{ variance } [\text{fem (male) x host}] + 9.6612 \text{ variance (male x host)} + Q \text{ (host)}. \]

\[ V_{\text{male x host}} = \text{Variance (error)} + 5.2443 \text{ variance } [\text{fem (male) x host}] + 10.489 \text{ variance (male x host)}. \]

\[ V_{\text{[fem (male) x host]}} = \text{Variance (error)} + 5.3158 \text{ variance } [\text{fem (male) x host}]. \]

Where, \( V_{\text{male}} = \text{Variance due to male parent}; \)

\( V_{\text{fem (male)}} = \text{Variance due to female parent}; \)

\( V_{\text{host}} = \text{Variance due to host}; \)

\( V_{\text{male x host}} = \text{Variance due to interaction between male parent and host}; \)

\( V_{\text{[fem (male) x host]}} = \text{Variance due to interaction between female parent and host and} \)

\( Q = \text{constant}. \)
Equations used to calculate the observed components in variance components estimation for the temperature experiment.

\[ V_{\text{male}} = \text{Variance (error)} + 4.4882 \text{ variance } [\text{fem (male) } \times \text{ temp}] + 8.9763 \text{ variance } (\text{male } \times \text{ temp}) + 13.465 \text{ variance } [\text{fem (male)}] + 26.929 \text{ variance } (\text{male}). \]

\[ V_{\text{fem (male)}} = \text{Variance (error)} + 4.504 \text{ variance } [\text{fem (male) } \times \text{ temp}] + 13.512 \text{ variance } [\text{fem (male)}]. \]

\[ V_{\text{temp}} = \text{Variance (error)} + 4.3501 \text{ variance } [\text{fem (male) } \times \text{ temp}] + 8.7001 \text{ variance } (\text{male } \times \text{ temp}) + Q \text{ (temp)}. \]

\[ V_{\text{male } \times \text{ temp}} = \text{Variance (error)} + 4.6345 \text{ variance } [\text{fem (male) } \times \text{ temp}] + 9.269 \text{ variance } (\text{male } \times \text{ temp}). \]

\[ V_{[\text{fem (male) } \times \text{ temp}]} = \text{Variance (error)} + 4.6595 \text{ variance } [\text{fem (male) } \times \text{ temp}]. \]

Where, \( V_{\text{male}} = \text{Variance due to male parent}; \)

\( V_{\text{fem (male)}} = \text{Variance due to female parent}; \)

\( V_{\text{temp}} = \text{Variance due to temperature}; \)

\( V_{\text{male } \times \text{ temp}} = \text{Variance due to interaction between male parent and temperature}; \)

\( V_{[\text{fem (male) } \times \text{ temp}]} = \text{Variance due to interaction between female parent and temperature and} \)

\( Q = \text{constant}. \)
Appendix 2

Modified Falconer’s formula used in the estimation of causal components in variance components estimation for both host switching and the temperature experiments (adapted from Liu, 1998).

\[ V_{\text{male}} = 2 \left( V_{\text{male}} \right), \]
\[ V_{\text{fem (male)}} = 2 \left( V_{\text{fem (male)}} - \frac{1}{2} V_{\text{male}} \right), \]
\[ V_{\text{male x host}} = 2 \left( V_{\text{male x host}} \right), \]
\[ V_{\{\text{fem (male) x host}\}} = 2 \left( V_{\{\text{fem (male) x host}\}} - \frac{1}{2} V_{\text{male x host}} \right), \]
\[ V_{\text{male x temp}} = 2 \left( V_{\text{male x temp}} \right), \]
\[ V_{\{\text{fem (male) x temp}\}} = 2 \left( V_{\{\text{fem (male) x temp}\}} - \frac{1}{2} V_{\text{male x temp}} \right), \text{ and} \]
\[ V_{\text{error}} = V_{\text{error}} \left[ V_{\text{fem (male)}} - \frac{1}{2} V_{\text{male}} \right]. \]

Where, \( V_{\text{male}} = \) Variance due to male parent;
\( V_{\text{fem (male)}} = \) Variance due to female parent;
\( V_{\text{male x host}} = \) Variance due to interaction between male parent and host;
\( V_{\{\text{fem (male) x host}\}} = \) Variance due to interaction between female parent;
\( V_{\text{male x temp}} = \) Variance due to interaction between male parent and temperature;
\( V_{\{\text{fem (male) x temp}\}} = \) Variance due to interaction between female parent.