

LIFE HISTORY, HOST CHOICE AND BEHAVIORAL PLASTICITY OF *Trichopria nigra*,  
(HYMENOPTERA: DIAPRIIDAE), A PARASITOID OF HIGHER DIPTERA

By

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To my father, the first and greatest scientist in my life.

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Abstract of Thesis Presented to the Graduate School  
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By

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*Trichopria nigra* (Nees) (Hymenoptera: Diapriidae) is a gregarious pupal endoparasitoid of several common fly species. Furthermore, it is a parasitoid of two of the most common pest fly species in North America: the stable fly *Stomoxys calcitrans* (L.), and the house fly *Musca domestica* L. *T. nigra* was first established in a North American laboratory from specimens that emerged from stable fly pupae collected in Russia and Kazakhstan in 1999. Little is known about the life history and definitive host range of this insect. No records of this insect in North America have been made. Its ability to successfully parasitize multiple common pest fly species, however, as well as its small size and inexpensive, simple rearing methods make it a potentially valuable biological control agent against stable flies and house flies.

The first photographs of *T. nigra* are presented with a detailed analysis of adult external morphology and sexual dimorphism. The rearing methods used in maintaining colonies of this parasitoid are provided. Dissections of *T. nigra* ovarioles were made to determine mean number of ova. Weights of, and head capsule widths from, parasitoids reared on two different hosts were made to determine whether variance in body size exists with regard to host size, and it was determined that a larger host does produce, on average, larger parasitoids of this species.

A longevity experiment was conducted to determine the mean lifespan of male and female parasitoids when provided with honey, water and host pupae. It was determined that providing honey lengthened adult lifespan, an effect that was increased when host pupae were reared in conjunction with honey. Providing hosts in the absence of honey and water led to the shortest lifespan for male and female parasitoids.

Two variations of a choice test were conducted, one in which adult parasitoids were conditioned for 48 h on the pupae of one of three host species, and another in which wasps were reared on two host species. Conditioning parasitoids for 48 h significantly increased the proportion of female wasps that chose that host species to which they were conditioned in an open-arena assay for house fly-conditioned parasitoids only. Rearing parasitoids on a particular host species led to a significant difference in host choice in an open-arena assay, with parasitoids strongly preferring to oviposit in the host species in which they had developed.

Y-tube olfactometer experiments were conducted to corroborate findings from the choice test experiments as well as to determine whether response of adult female parasitoids changed significantly with age and previous exposure to a host insect. Conditioning parasitoids increased the likelihood, compared to unconditioned controls, of females choosing their conditioning host when presented with two choices in a Y-tube olfactometer. Rearing parasitoids on a host species greatly increased the likelihood of females choosing their natal host when presented with two choices. Lastly, it was determined that the strength and speed of female response to host odors does not significantly change in the first five days of adult emergence, although the most number of females responded, and demonstrated the fastest response, between two and three days post-emergence.

## CHAPTER 1 INTRODUCTION

Because the hosts of many parasitic wasps are flies commonly considered pests of humans and livestock, the significance of parasitoids as biological control organisms is considerable (Rueda and Axtell 1985, Anderson and Leppa 1992). Two of the most important pest flies with regard to human health, well-being and economics in North America are often considered *Musca domestica* L., the house fly, and *Stomoxys calcitrans* L., the stable fly. Other flies which are considered pests of humans and commercially-reared animals include the face fly *Musca autumnalis* De Geer, the horn fly *Haematobia irritans* (L.), and a variety of blow flies and flesh flies in the families Calliphoridae and Sarcophagidae.

One of the primary motives for fostering research in the field of biological control is the growing resistance of pest insects to chemicals utilized in controlling them. House flies were among the first insects to demonstrate documented resistance to insecticides, as DDT became commercially available for house fly control in 1944. By 1947, DDT had failed to control fly populations in Europe (Decker and Bruce 1952). House fly resistance to pyrethroids emerged within ten years of introduction of these chemicals as commercial products for house fly control (Hogsette 1998). Resistance of insects has long been known to develop swiftly in insect populations subjected to frequent, and high-dosage, pesticide applications (Mallis et al. 2004). An additional dimension to the problem of pest fly resistance to insecticides is the apparent genetic basis for pesticide resistance exists in the house fly, which is allelic in nature and subject to both genetic mutation and the migration of pest fly populations with novel alleles (Rinkevich et al. 2007). In the future, agriculturists may find that relying solely on pesticidal methods to prevent insect damage to livestock and crops will have become wholly ineffective. Currently it proves impossible (Hogsette 1998) to maintain livestock pests such as horn flies at or below their

damage thresholds with registered pesticides. Treating agricultural pest problems with biocontrol organisms, however, does not incur resistance to that treatment method as do pesticide applications.

In light of the reduced efficacy of traditional chemical control and the effects of pesticides on the environment, there exists an increasing need for novel and effective biological control organisms which can be used to reduce the number of pest insects and their effects on humans and agricultural products. The aims of this research were to better understand the biology and behavior of a species of endoparasitic wasp, *Trichopria nigra* (Nees) (Hymenoptera: Diapriidae), which parasitizes numerous species of flies commonly considered pests of humans and livestock.

Because the facility in which this research was conducted is the first known location of in-laboratory rearing of *T. nigra*, a detailed identification of this species, along with this researcher's rearing methodology, is included (Chapter 3). A longevity experiment was conducted to determine the longevity of this species when presented with a food source, water, and hosts in several combinations, as well as to compile a greater understanding of the life history of this species (Chapter 4). Additionally, two experiments were performed to determine (1) the preference of this species when provided with multiple choices for a host, and whether that choice can be influenced (Chapter 5); and (2) whether olfaction is an important method this species uses to locate a host, and if the strength of an olfaction-based host choice changes with the age of the adult insect (Chapter 6).

## CHAPTER 2 LITERATURE REVIEW

### **Evolution of the Parasitic Hymenoptera**

Within the order Insecta, parasitic life history strategies account for the second-most popular life history strategy in insects after phytophagy (Althoff 2003). Within Hymenoptera, parasitism of other insects is so prevalent across the families of waisted wasps (Hymenoptera: Apocrita) that an early evolutionary origin of parasitic Hymenoptera is likely (Whitfield 1992). Furthermore, the prevalence of parasitic lifestyles – with the majority of families of Apocrita possessing genera that are considered parasitoids of plants or other insects – indicates that such life history strategies are highly successful.

Systematic coevolution of insects and the plants and animals which provide them with food, shelter and reproductive niches is well-documented across taxa. A common entomological example is louse-host specificity (Bush and Clayton 2006). Parasitoid-host coevolution is similarly apparent – at least among plant parasitoids. Sidhu (1984) observes that genetic selection in many plants important to humans (and to insects as hosts) is the result of breeding and crop selection. The evolution of host animals, in contrast, is often driven more by ecology and behavior, making closely-related coevolutionary changes more difficult to observe. However, diversity of parasitoids seems, generally, to be under the control of host diversity (Sidhu 1984, Hufbauer 2001).

According to Quicke (1997), the fossil record contains preserved hymenopteran fossils dating as early as the mid-Triassic period. There are currently two extant suborders of Hymenoptera (Johnson and Triplehorn 2004): the Symphata (including sawflies and wood wasps) and the Apocrita (all other hymenopterans including all known parasitoids), although the outset of the Jurassic period saw diversification of Hymenoptera to include the extinct suborders

Karatavidae and Ephialtitidae (Quicke 1997). The appearance of morphological features that would allow for parasitism of a host, such as a lengthened ovipositor, hint at the emergence of the first parasitoid hymenopterans during the Jurassic period.

Within Hymenoptera, the suborder Apocrita is primarily defined (Johnson and Triplehorn 2004) by the fusion of the first abdominal segment to the thorax and the constriction or “waist” between the first and second abdominal segments. Other defining characteristics, such as the number of basal hindwing cells (Quicke 1997), further separate Apocrita from the evolutionarily primitive sawflies. Within Apocrita, ten of the twelve superfamilies largely include parasites of either plants (gall wasps, fig wasps) or animals (overwhelmingly other members of Insecta) (Johnson and Triplehorn 2004). Superfamilies within Hymenoptera which are known to contain parasitoid members include Ichneumonidea, Chalcidoidea, Evanioidea, Proctotrupoidae, Bethyloidea, Scolioidea and Vespoidea (Whitfield 1998, Johnson and Triplehorn 2004).

The question as to why parasitism evolved across multiple insect orders has been under speculation for decades (Quicke 1997). Parasitism most likely evolved only once in basal Hymenoptera (Eggleton and Belshaw 1992) as, driven by competition for food sources, early mycophagous wasps began to kill larvae already feeding on fungi and superimpose their own larvae on the food source. The most primitive (from a cladistic viewpoint) parasitic superfamilies – the Ichneumonidea and Evanioidea – are often found in close relation to detritus and fungal-heavy environments (Eggleton and Belshaw 1992, 1993); the more advanced parasitoid hymenopterans practice life history strategies ranging from parasitizing developing larvae to take advantage of a longer developmental time (Gelman et al. 2005b), to drilling into host puparia and laying eggs outside the host. Further discussion on the advantages and disadvantages of a parasitic lifestyle in Hymenoptera follows below.

From an evolutionary viewpoint, it might be assumed that if parasitism evolved once in a clade and was not only retained as a life history strategy but became the overwhelming life history strategy of an entire suborder (Borror et al. 1974, Johnson and Triplehorn 2004), that life history strategy must offer distinct and significant benefits to the insects practicing it. One advantage that parasitoids encounter is that they do not have to devote time as immature insects to foraging for, locating, and processing food (Quicke 1997). Parasitoid wasps live within their source of food, water, and (often) endocrine compounds (Gelman et al.. 2005a). Their hosts confer protection from changes in temperature and humidity, and unless the host insect is predated upon, the parasitoids are safeguarded from predation themselves.

Obvious risks are associated with a parasitic life history for parasitoid hymenopterans. The quality of the host often determines the quality of the next generation of parasitoids (Ellers et al. 1998, Consoli and Vinson 2004), as the available nutrients for the developing parasitoids are limited by the size and carrying capacity of the host insect. Another drawback to parasitism is the risk of choosing a host species that is poorly suited to parasitism, especially in non-specific solitary parasitoid wasps (Quicke 1997, Ferrero 2006 from unpublished research).

### **Biological Control of, and Economic Damages Caused by, Pest Flies**

Because the hosts of many parasitoid wasps are flies commonly considered pests of humans and livestock, their significance to human populations is considerable (Rueda and Axtell 1985, Anderson and Leppla 1992). The most important pest flies with regard to human health, well-being and economics in North America are often considered *Musca domestica* L., the house fly, and *Stomoxys calcitrans* (L.), the stable fly (Geden and Hogsette 1994). Other flies which are sometimes considered pests of humans and commercially-reared animals include the face fly, *Musca autumnalis*, the horn fly, *Haematobia irritans*, the flesh fly, *Sarcophaga spp.*, and the black garbage fly, *Ophyra spp.*

According to the USDA, confined and range cattle contributed the largest share of agricultural profit in the United States with 37.9 billion dollars in income (Geden and Hogsette 1994). Of this amount, 17.6 billion dollars in damage come from confined cattle facilities; the highest infestations of pest flies which are hosts of numerous parasitoid species occur in confined cattle facilities. While traditionally considered pests of confined cattle only (Smith and Rutz 1991), stable flies have been known to disturb cattle in open fields in geographic locations that are subject to periodical rainfall (Geden and Hogsette 1994). Directly, stable flies impact cattle by blood-feeding, which reduces overall health and feeding behavior of the animal and, ultimately, quality of product (Campbell and Berry 1989).

Poultry contribute a large portion of income to the agricultural industry in the United States, and with an increase in poultry rearing in the past five decades from small agribusinesses to large-scale poultry rearing facilities, pest flies have become an increasingly problematic issue. The primary pest of poultry agribusinesses, house flies, present a sanitation and public health problem (Mann et al. 1990). Transmission of avian pathogens as well as bacteria known to cause human illness (Geden and Hogsette 1994) create a potentially hazardous working environment in poultry houses. The house fly has been implicated as a potential vector of pathogens that pose serious risks to human health (Eldridge and Edman 2003) such as poliomyelitis, cholera, typhoid, tuberculosis and dysentery. Additionally, house flies swarming in confined spaces such as rearing facilities causes an annoyance such that poultry farms may incur a monetary loss in employee turnover, reduced efficiency of work, and time spent implementing control methods for pest flies in response to complaints from public health agencies.

Insect resistance to chemical control is another justification for the importance of parasitoids in improving agricultural and domestic aspects of human life. Insect physiological

resistance to chemical control has, since the implementation of en masse pesticide application in the forties, become a growing problem for agriculturalists worldwide (Learmount et al.. 2002). Hogsette (1998) documents that house fly resistance to pyrethroids emerged within ten years of implementation; resistance of pest filth flies to avermectins such as abamectin (Clark et al.. 1995) developed swiftly in insect populations and established readily in the gene pool. In the future, agriculturists may find that relying solely on pesticidal methods to prevent insect damage to livestock and crops will have become wholly ineffective, even as currently it proves problematic (Hogsette et al. 1991) to maintain livestock pests such as horn flies at or below their damage thresholds with registered pesticides. Treating agricultural pest problems with biocontrol organisms, however, does not incur resistance to that treatment method as do pesticide applications.

Most commercially-utilized biocontrol organisms are wasps which are pupal parasitoids parasitoid in the family Pteromalidae (Hymenoptera: Chalcidoidea). Biological control of pest flies involves releasing either adult parasitoids of the pest fly, or fly pupae with developing parasitoids within, en masse at an infested location (Geden and Hogsette 2006). Adult parasitoids increase the mortality of the host insect at a specific point in development by killing the host, usually prior to adult eclosion or adult emergence from the puparia (Rueda and Axtell 1985, Quicke 1997). Little is known about the efficacy of other parasitoid families in controlling pest flies. *Trichopria nigra* (Hymenoptera: Diapriidae) is a gregarious endoparasitoid of pest fly pupae that has been shown to significantly reduce population levels of house fly and stable fly pupae in the research conducted within this body of work. It is this researcher's goal to demonstrate that *T. nigra*, a novel and little-known parasitoid wasp is an efficient biocontrol

organism for the stable fly *S. calcitrans*, and potentially of other pest fly species within its host repertoire via selective conditioning.

## **Family Diapriidae**

### **Biology**

The family Diapriidae (superfamily Proctotrupeoidea) consists of minute wasps, all of which are parasitoids of other insect taxa (Nixon 1980). Diapriid wasps are known endoparasitoids of members of Diptera, Coleoptera (Masner 1993), and in the unique case of genus *Ismarus*, other Hymenoptera (Loiacono 1987). Diapriidae is divided into four subfamilies: Ismarinae, Ambositrinae, Belytinae and Diapriinae. Some members of Diapriidae are solitary; many, such as *Trichopria nigra*, are gregarious endoparasitoids (Nixon 1980). Solitary parasitoids are defined as those insects which lay one egg per host; gregarious parasitoids lay multiple eggs per host (Johnson and Triplehorn 2004). Subfamily Diapriinae, to which *T. nigra* belongs, shares the fewest traits and is often considered by insect taxonomists as the subfamily to which parasitoids not fitting the other three subfamilies are assigned (Buss, personal correspondence).

Medvedev (1988) provides a thorough catalog of characteristics shared among members of Diapriidae. Among these features include a dark and glossy exoskeleton, a small and rounded head with long antennae, and a markedly narrow first abdominal segment (petiole). Despite most members of Diapriidae having large wings relative to absolute body size, they are poor fliers compared to many other Hymenoptera (Medvedev 1988).

### **Distribution**

While genera of Diapriidae are distributed worldwide, (Masner 1976, Masner 1993), the exact distribution of most of the approximately four thousand species placed within Diapriidae is largely unknown (Masner and García 2002).

## *Trichopria nigra*

### **Origin and Distribution**

Roger Moon (University of Minnesota) collected pupae of *Stomoxys calcitrans* on dairy farms near the cities of Almaty, Kazakhstan, and Kraznodar, Russia, in 1999 (C.J. Geden, USDA/ARS, personal correspondence). The purpose of such collections was to discover novel parasitoids of the stable fly and house fly. Pupae collected by Dr. Moon were transported to the United States and the first documented emergence and colony establishment of *T. nigra* occurred in 2000 at the USDA/ARS CMAVE facility in Gainesville, Florida. This is the first documented laboratory colony of *T. nigra*.

The distribution of *T. nigra* is not completely known; its discovery in Eastern European and Eurasian sites by Dr. Moon suggests that distribution is at least partially Palearctic; Medvedev (1988) cites the distribution as Romania (Moldavia). The most recent record of *T. nigra* in the wild is a 2002 collection of *Trichopria* spp. taken from sites in Kalambaka, Greece (Petrov 2002); the collector lists the known distribution of *T. nigra* as Germany north to Sweden and east to Moldova. Given the proximity of Moldova and Sweden to Russia, it is likely that the distribution of *T. nigra* extends from central Europe into northern Asia.

Related species in the genus *Trichopria* are distributed worldwide; collections have been made in the eastern (Bradley et al. 1984) and western United States (Krombein et al. 1979a; Krombein et al 1979b), as well as European countries such as Hungary (Hogsette et al. 1994). In Africa, *Trichopria* spp. have been observed in countries such as Zimbabwe (Huggert and Morgan 1993; Morgan et al. 1990) and Ethiopia (Huggert 1977).

Given that many discoveries of *Trichopria* species are made in and near poultry houses on all continents the genus has been discovered (Hogsette et al. 1994; Morgan et al. 1990), and that *T. nigra* emerged from *S. calcitrans* pupae collected by Dr. Moon on dairy farms, it is likely that

*T. nigra* may live and reproduce at least in the proximity of livestock- and poultry-rearing facilities.

## **Biology**

The first entomological description of *Trichopria nigra* is attributed to Nees (1834); this species was included in Kieffer's 1916 taxonomic treatise on Diapriidae (Kieffer 1916), and more recently in Masner and García's (2002) description of Diapriidae. Few taxonomic keys provide an adequately detailed identification of the genus. Medvedev (1988) offers the following couplet for identification of members of *Trichopria*:

...Prothorax with collar of white or silvery hairs; collar broader than 1<sup>st</sup> antennal segment, and these hairs discumbent. Temples with white or silvery hairs. Longitudinal axis of eye usually not shorter than length of middle coxa. Head roundish or rectangular on dorsal surface. *Females*: Antennae at least with dark clava. Antennal segments, commencing from the 3<sup>rd</sup>, round...

In addition to the above couplet, *T. nigra* adults possess features that distinguish the species from sister species; the 2<sup>nd</sup> and 3<sup>rd</sup> antennal segments are approximately equal in length and the legs, in contrast to the glossy black body color, are a dark yellow color and nearly translucent (Medvedev 1988). Males and females are dimorphic with respect to antennal shape; females possess a clubbed terminus at the distal end of each antenna whereas males possess comparatively more uniform antennae. The sexes are not dimorphic with respect to size, although a larger host (e.g., *S. bullata* is larger than *S. calcitrans*) will usually produce larger parasitoid offspring of both sexes (see Chapter 3: Effect of Host Size on Parasitoid Size).

## **Food and Hosts**

*T. nigra* has not, to the author's knowledge, been observed feeding in the wild. A related species, *T. stomoxydis*, which is a gregarious endoparasitoid of *S. calcitrans*, shares a similar life history to *T. nigra*. It was determined that *T. stomoxydis* does not host feed prior to, or following,

host location and oviposition; without a food source such as plant nectar, longevity was calculated to be approximately three days (Morgan et al. 1990). Other shared life history features include the endoparasitic nature of larval development; both *T. nigra* and *T. stomoxydis* immatures feed and develop within the body of the pupating host. Unlike *T. nigra*, however, *T. stomoxydis* is host-specific to *S. calcitrans* (Nash 2005).

In colony at the USDA/ARS CMAVE facility, adult *T. nigra* are provisioned with honey on an *ad libitum* basis. Despite being a koinobiont species, adult female *T. nigra* do not host-feed and thusly do not require a meal to complete ovariole maturation (Chan and Godfray 2005). Water is also provided *ad libitum*. Although it is likely that this insect procures moisture from nectar food sources in the wild; the moisture content of most commercial honeys, at 20-40% is considerably lower than that of plant nectars due to the concentration of sugars (Bijlsma et al. 2006). For a detailed description of rearing methodology, see Chapter 3.

CHAPTER 3  
NOTES ON SPECIES RECOGNITION AND REARING METHODS

**Introduction**

Very few references to *Trichopria nigra* (Nees) exist in the scientific literature. A 1984 collection of seaweed fly pupae from two species collected from Kieler Forde, Germany (Heitland 1988), *Coelopa frigida* (F.) and *Fucellia tergina* (Zett.), yielded specimens identified as *T. nigra*. Petrov (2002) mentions *T. nigra* in a catalogue of Diapriid parasitoids found in Greece, although first identification of this species is attributed to Nees in 1834 (noted in the Hymenoptera collection of the Zoological Museum, University of Copenhagen). A field survey by Hogsette et al. (1994) in central Hungary lists two species of *Trichopria* which were found in proximity to pupae of *Musca domestica* L. and *Stomoxys calcitrans* (L.); given the discovery of *T. nigra* in Russia and Kazakhstan by Moon (personal communication) as well as in China by Yujie et al (1997), it is possible that this species has a broadly Palearctic range.

The lack of a definitive and readily available species description, in addition to the lack of known photographs and images of this insect, indicate the need for a detailed analysis of *T. nigra*'s morphology and differentiation from related species. Furthermore, rearing methodology of this insect is included for the benefit of scientists who wish to rear *T. nigra* in a laboratory setting. To this researcher's knowledge, both strains of *T. nigra* (the Russian-collected population and the Kazakhstan-collected population) are morphologically and behaviorally similar, and are reared identically.

**Positive Identification of Species**

**Adult Insect**

*T. nigra* adults share many of the general traits of family Diapriidae, being described as small (1-6 mm), black parasitoid wasps with large eyes and geniculate antennae possessing 11-

15 segments, and an ovipositor that is not visibly protruding (Masner and García 2002). The insect is readily identified as a wasp, with three well-defined body segments, a narrow waist, and two pairs of wings. The sexes are not sexually dimorphic with regard to size, as both male and female adults grow to a maximum mean length of 2-3 mm from head capsule to terminal abdominal segment.

It has been documented (Ueno 2004) that the size of many pupal parasitoids, as well as the sex ratio of progeny produced, varies in direct relation to the size of their hosts. The sizes of *T. nigra* offspring reared on two host fly species' pupae of different sizes were analyzed to determine if size plasticity with regard to host size is correlated in this parasitoid species. Adult *T. nigra* reared on pupae of *Stomoxys calcitrans* (L.) were on average one-third lighter (mean mass  $0.069 \pm 0.001$ mg.) than individuals reared on pupae of *Sarcophaga bullata* Parker (mean mass  $0.106 \pm 0.001$ mg.), with a head capsule diameter that is ~80% that of individuals reared on *S. bullata* (Table 3-2).

The head is rounded and possessing of large, black eyes that are the most prominent feature of that segment. Antennae attach superiorly and medially to the eyes, and sexing of the adult insect is most easily performed by examining the shape of the antennae. In female insects the antennal segments enlarge distally into a distinct club (Figure 3-2). Male *T. nigra* adults, however, possess antennae that are nearly uniformly filamentous (Figure 3-3). Both male and female insects possess, as do all Diapriine wasps (Masner 1976), thirteen antennal segments. Second and third antennal segments are equal in length; this feature along with the unique coloration of the legs is considered the defining trait for *T. nigra*, separating it from *T. socia* in a dichotomous key (Medvedev 1988).

The exoskeleton is uniformly black in color and highly reflective, almost lustrous; the legs are of dark amber to golden yellow color, with femoral and coxal segments distinctly darker than the tarsal segments. The entire insect is sparsely covered with slender bristles. The cephalic point of the first thoracic segment, as well as thoracic segments 2, 3 and the thoracic-abdominal juncture, are covered in dense collars of short, golden hairs.

*T. nigra* ovipositor size is small in relation to body length, and markedly short in comparison to ovipositor length in taxa such as Ichneumonidae. The length of the ovipositor is retracted except during probing and oviposition (Figure 3-4). This feature is shared among Diapriidae (Medvedev 1988). Females are gregarious in oviposition behavior, and adult *T. nigra* chew multiple exit holes in the host puparia (Figure 3-5A). Developing parasitoids require most of the room inside the developing fly host, and dissected puparia reveal an empty “mummy” or cuticle which protected the parasitoid larvae inside (Figure 3-5B).

### **Female Reproductive Potential with Respect to Body Size**

Female reproductive anatomy of *T. nigra* consists of two bi-lobed ovarioles (Figure 3-6) of the meroistic type, germ cells differentiating into both oocytes as well as nurse cells. Like most koinobionts, female *T. nigra* emerge with competent (autogenous) oocytes (Waage and Greathead 1986) and do not need to feed in order to oviposit, and oocytes are likely not replenished as they are used. As demonstrated in Figure.3-1, 3-2, and 3-3, adult body size can vary greatly in this species, to the extent that it is questioned whether the size of adult female *T. nigra* is indicative of their reproductive potential.

To determine whether a correlation exists between adult female size and fecundity (egg load), dissections of female *T. nigra* reared on two hosts were conducted. Twenty 2-5 day old female *T. nigra* reared on *Stomoxys calcitrans* and twenty 2-5 day old female *T. nigra* reared on *S. bullata* were ovariectomized. The number of eggs for each female was counted (Figure 3-6),

and the width of the head capsule measured. The mean ( $\pm$ SE) head widths and number of eggs are listed in Table 3-1. A regression analysis was conducted for each treatment as well as both treatments combined, with head capsule width and number of eggs as variables (Figure 3-7). Furthermore, PROC GLM was conducted (SAS 2000) to determine whether rearing host has an effect on egg load.

For females reared on *S. calcitrans* pupae, the average head capsule width was 0.320 mm, approximately 84% of the head capsule width of females reared on *S. bullata* pupae (0.380 mm) (Table 3-1). This difference was statistically significant (SAS, 2000) and was consistent with hypotheses about the *T. nigra* size/fitness relationship, and previous parasitoid research (see Quicke 1997; Cohen et al. 2005). Because head capsule width is an indicator of overall body size and often, of fecundity and adult fitness (Liu 1985, Visser 1994, respectively), it was expected that larger females (i.e., females reared on *S. bullata*, the larger host) would possess more eggs. This was found to be the case as well, with *T. nigra* reared on *S. calcitrans* possessing on average approximately 76% of the egg load their *S. bullata* cohorts developed.

The observed differences in body size and fecundity of *T. nigra* when reared on two hosts of differing host sizes agrees with literature on parasitoid fitness with respect to host fitness. In general, parasitoids whose hosts are larger have more food available and therefore can acquire more nutrition for growth and development (e.g., of eggs). Some literature suggest that for koinobiont parasitoids, who develop internally and allow their hosts to continue physiological development, the correlation between host size and parasitoid fitness is not necessarily valid (see Jenner and Kuhlmann 2005). For *T. nigra*, however, which is an unusual koinobiont in that it parasitizes its hosts during their pupal stage, host growth in a spatial context has mostly ceased and in fact is constrained by the puparium containing the host. It may be assumed that a clearly-

lineated relationship between body size and size of the host is a characteristic more commonly associated with idiobionts and those koinobionts which parasitize later stages of their hosts.

### **Rearing Methods**

At 25°C, development requires ~26 d from oviposition for adult emergence (personal observation). Males and females emerge from puparia at the same time. It is not known whether adults are competent to oviposit immediately following adult emergence. It is also not known whether any mating occurs inside host puparia although such mating would prove unlikely given the spatial constraints of adult parasitoids inside the puparia. A related species, *Trichopria stomoxydis*, mates shortly after emergence of adults from host puparia, with male insects emerging approximately 24 h prior to female insects (Morgan et al. 1990). It is likely that *T. nigra* follows a similar mating behavior. For a detailed life history of *T. nigra*, see Chapter 4.

In this researcher's laboratory, *T. nigra* has successfully parasitized pupae of *Stomoxys calcitrans* (L.), *Ophyra aenescens* (Wiedemann), *Haemotobia irritans* (L.), and *Sarcophaga bullata* Parker. Colonies of *T. nigra* have been successfully maintained utilizing *S. bullata* and *S. calcitrans* as hosts. Additionally, *T. nigra* females readily examine and probe with their ovipositors the pupae of *Musca domestica*, and have been observed ovipositing inside *M. domestica* puparia; regardless of a high pupal mortality in the host, no adult parasitoids have emerged in numerous laboratory rearing attempts (personal observation). Other instances of *T. nigra* parasitizing a dipteran host include the cheese skipper, *Piophilina casei* L. (Teodorescu and Ursu 1979). All of the above hosts are flies in the infraorder Muscamorpha. Additionally, *T. nigra* has been reared on the small pest fly *Sturmia bella* (Meigen) (Diptera: Tachinidae) (Nash 2005). The majority of demonstrated *T. nigra* hosts are commonly considered pest flies in close association with human habitats; as data on this species is highly incomplete a detailed list of all hosts is not available.

## **Containers**

Clear plastic storage containers (ca. 25 x 14 x 12 cm) were utilized as rearing containers. Container tops were discarded and the containers sealed across their opening with a white cotton tube and two rubber bands to prevent wasp escape and to allow for periodic access to the container (Figure 3-8). Rearing containers were placed on one side to that access via cotton tube was from the side rather than the top of the container. Water was provided *ad libitum* on cotton balls placed in one-ounce plastic cups (Sweetheart Cup co., Owings Mills, MD); one or two water-soaked cotton balls in cups were placed in each rearing container. When access to the interior of the container was not required, the end of each cotton tube was tied securely into a single knot.

## **Diet**

Commercially-purchased (human consumption grade) honey was provided to adult wasps on cotton balls placed in two-ounce plastic food service containers (Sweetheart Cup co., Owings Mills, MD). Additionally, a square piece of muslin cloth (ca. 10 x 6 cm) was covered in a thin layer of honey and placed on the inside superior wall of the rearing container. This was done to prevent drowning of parasitoids in the honey due to their small size.

Since *T. nigra* does not host feed, no pupae as a protein food source were required for colony maintenance. See Chapter 4 for a discussion on whether honey is required for colony maintenance. This researcher has also successfully reared *T. nigra* on a 50% v/v solution of honey and water provided on cotton balls in one-ounce plastic food service containers (Sweetheart Cup co., Owings Mills, MD).

## **Host Provisioning**

Both strains of *T. nigra* (Russian and Kazakhi,) were reared on ~2-day old pupae of *Sarcophaga bullata* in ~300 cc paper containers covered loosely with a piece of muslin cloth

and a rubber band. Paper containers of stung pupae were covered to prevent accidental hyperparasitism from other species reared in the laboratory, and kept in an incubator at 25° C until adult emergence was first noted. Time required for development, from stinging of pupae to emergence of adults, was approximately 26 days at a temperature of 25° C. At first sign of emergence from puparia, adults were transferred to rearing containers (see above) by placing the paper food service containers containing pupae and new adults, uncovered, in the rearing containers. Adult wasps, beginning at 5-6 d post-emergence, were provisioned with ~250 cc fresh unstung 2- to 3-day old *S. bullata* pupae for 5-6 days on a weekly basis, to establish the subsequent generation. For the purpose of experimentation requiring *T. nigra* reared on hosts other than *S. bullata*, host provisioning methods were identical except that ~250 cc of pupae from *S. calcitrans* or *M. domestica* were utilized.

An interesting aspect of host provisioning for *T. nigra* is that, while adult females will readily attempt to parasitize *M. domestica* pupae, and very few host pupae survive to adult emergence, no parasitoids emerged from puparia. The mechanism for this phenomenon is unknown, although it is hypothesized that rapid melanization of wounds caused by probing kill parasitoid eggs, if any are laid (personal observation). Dissections of house fly puparia stung by *T. nigra* at 2, 5 and 10 days post-sting have not yielded any parasitoid larvae.

Table 3-1. Head capsule widths and egg numbers of *Trichopria nigra* females and males (50/50 sex ratio) reared on pupae of *Sarcophaga bullata* and *Stomoxys calcitrans*. For *S. bullata* reared, F=8.99; df= 1 P<0.05. For *S. calcitrans* reared, F=8.60; df= 1 P<0.05.

Rearing Host	Head Capsule Width (mm)	Egg Load	<i>n</i>
<i>S. bullata</i> emerged	0.382 (.007)a	114.3 (6.4)a	20
<i>S. calcitrans</i> emerged	0.320 (.010)b	87.2 (4.5)b	20

Means in the same column followed by the same letter are not significantly different (SAS 2000).

Table 3-2. Head capsule widths and weights for female and male *Trichopria nigra* (50/50 sex ratio) reared on pupae of *Sarcophaga bullata* and *Stomoxys calcitrans*.

Rearing Host	Head Capsule Width (mm)	Weight (mg)
<i>S. bullata</i> emerged	0.384 (.005)	0.106 (.001)
<i>S. calcitrans</i> emerged	0.322 (.005)	0.069 (.001)



Figure 3-1. Adult female *Trichopria nigra*, lateral view.



Figure 3-2. Adult male *Trichopria nigra*, lateral view.



Figure 3-3. Probing female *Trichopria nigra*. The ovipositor is extended and inserted into the puparium of *Stomoxys calcitrans*.



Figure 3-4: Dissection of *T. nigra* abdomen reveals the retracted ovipositor in close relation to the ovarioles.

A)



B)



Figure 3-5. A) Empty puparium of *Sarcophaga bullata*. Multiple exit holes created by *Trichopria nigra* are visible. B) Dissected puparium of *S. bullata*. B) Dissected host remnants. During parasitoid development, the interior of the host fly is consumed.



Figure 3-6. Microscopy image (400x) of 3-day-old *Trichopria nigra* ovarioles. Small dark circles at the base of the ovarioles (arrow) are the spermathecae.

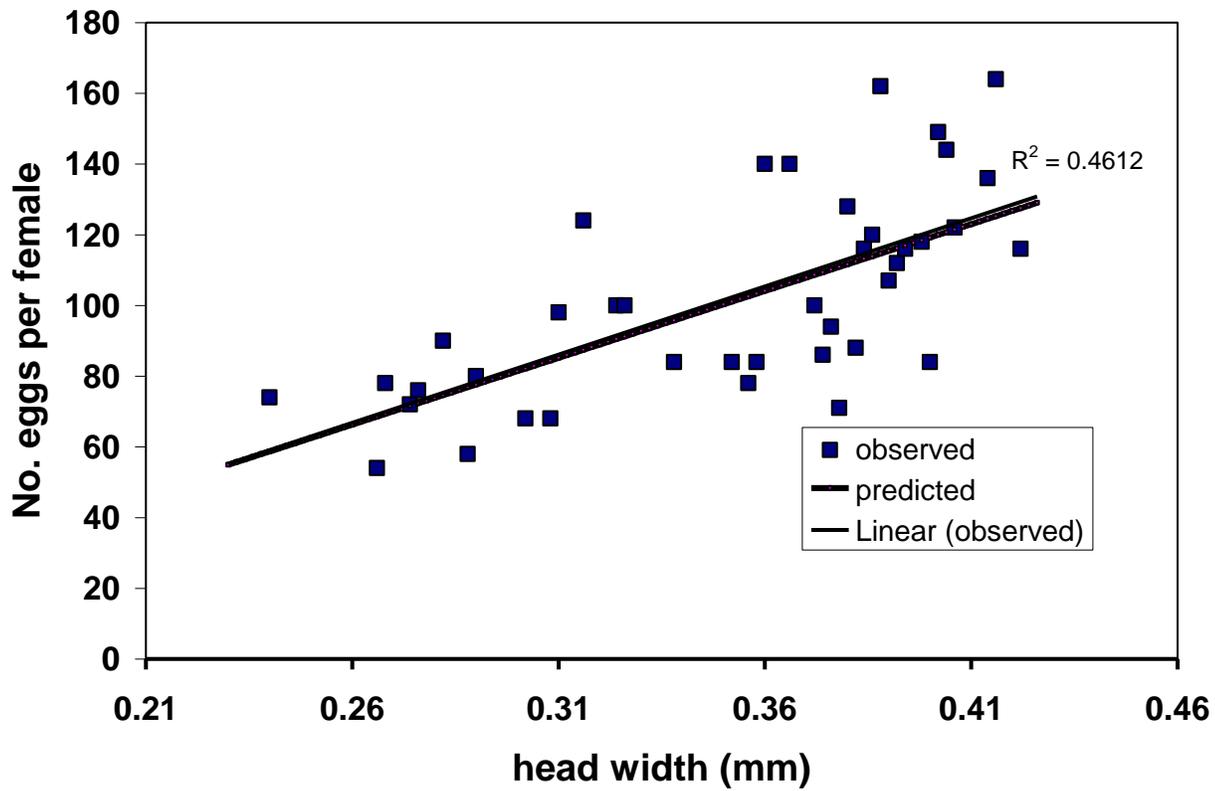


Figure 3-7. Regression analysis for body size of female *Trichopria nigra* (directly correlated to head capsule width in mm) and egg load size (in eggs/female).



Figure 3-8. Rearing containers used for colonies of *Trichopria nigra* at the USDA/ARS Center for Medical, Agricultural and Veterinary Entomology in Gainesville, FL.

## CHAPTER 4 LONGEVITY AND FECUNDITY EXPERIMENTS

### Introduction

Reproduction is nutritionally costly in all animals; insects prove no exception. Female parasitoids that oviposit must have competent eggs, and must be competent themselves to lay eggs. Many parasitoids, before laying eggs inside or on a host, will utilize a host as a food source (Godfray 1994). This feeding strategy of utilizing a potential host to feed both an adult parasitoid and its offspring is widespread among idiobionts. Females of idiobiont parasitoid species emerge without competent eggs and must host-feed to acquire protein for egg maturation. Additionally, odobionts parasitize their hosts in later host life stages (Quicke 1997). In contrast, koinobionts – parasitoids which parasitize their hosts early in development and allow hosts to continue development – rarely host-feed.

The life history of *Trichopria nigra* (Nees) is largely unknown. It has been observed that *T. nigra* does parasitize multiple species of higher flies, and that it is not observed to feed on potential hosts before or during oviposition. From an evolutionary perspective, these behaviors are unusual. *T. nigra* is a koinobiont parasitoid. Its hosts continue to develop post-oviposition (see chapter 3 for experiments and observations on this topic). Many of the characteristics of *T. nigra*'s reproductive behavior and physiology however, reflect those of idiobiont parasitoids (Hawkins et al. 1990); for example, idiobionts tend to have a relatively wide host range that encompasses multiple host species. Preliminary data indicate that *T. nigra* readily parasitizes several closely-related host species. Additionally, laboratory rearing of this insect has demonstrated a low female fecundity, despite gregarious immature development within the host (Geden, personal observation). Lastly, *T. nigra* parasitizes its hosts later in their development, a

trait normally ascribed to idiobiont species (Quicke 1997), yet is an endoparasitoid with larval wasps demonstrating a koinobiont trait by feeding and developing inside the host viscera.

When a parasitoid develops inside a host that is growing (such as a larva), nutritional resources are continually acquired by the host. Less need exists for a well-yolked, larger egg, because the host's continual growth provides a renewed food source (Giron et al. 2002). Because of this, koinobionts tend to possess smaller eggs and are proovigenic (i.e., eggs are chorionated and the female insect does not require a protein meal to produce yolk). In general, it is assumed that the main nutritional components gleaned from feeding on host hemolymph are sugars such as trehalose and amino acids (Giron et al. 2002, Quicke 1997), although results from longevity experiments (Ferracini et al. 2006, Jervis and Kidd 1991) offer inconclusive evidence that host-feeding imparts any benefits such as longevity to the maternal parasitoid.

Because the purpose of host-feeding is solely to develop the ovaries and produce competent eggs (Quicke 1997), it would not be expected that host-feeding incurs any lifespan-lengthening effects to female parasitoids. Therefore, *T. nigra*, a parasitoid which has never been documented host-feeding, should not benefit from the presence of host pupae with regard to lifespan because it would not be expected that such hosts would be utilized as a food source. However, it is not known whether *T. nigra* males and females, when provided with a carbohydrate food source such as honey or plant nectar, live longer or produce more progeny than individuals maintained solely with a source of water. Additionally, it is not known whether the presence of host pupae for the purpose of oviposition appreciably lengthens or shortens the lifespan of male and female *T. nigra*.

The effects of providing food and hosts to parasitoid wasps have been studied in many non-diapriid parasitoids. In an early experiment conducted on *Nasonia vitripennis* (Walker)

(Hymenoptera: Pteromalidae) it was determined that the longevity of unfed parasitoids was approximately 7 days for females and less than two days for males (Nagel and Pimintel 1963). It is generally assumed that for many insects the addition of an energy source (i.e., food) extends the lifespan of individuals. Various parasitoid species in the families Pteromalidae and Diapriidae which are reared at CMAVE, for example, live as long as four or five weeks in a colony situation, where rearing containers include a food source in the form of honey (Ferrero, personal observation).

Irvin et al. (2007) determined that when three species of egg parasitoids in the genus *Gonatocerus* were given a honey-water solution, the longevity of male and female parasitoids increased between 1400% and 1800% in all three species, compared to parasitoids given only water. Furthermore, studies which involved feeding parasitoids a variety of potential carbohydrate food sources in the form of insect-derived sugars (such as trehalose) or plant-derived sugars (such as fructose), indicate that plant-derived sugars increase the longevity of parasitoids more appreciably (Jacob and Evans 2000, 2004). The Diapriid parasitoid, *Trichopria stomoxydis* Huggert, provides compelling evidence that feeding on a carbohydrate source such as a sugar to parasitoids in a laboratory setting changes the lifespan of the adult insect. Morgan et al. (1990) claimed that *T. stomoxydis* presented little potential as a biological control agent in part because of its short adult lifespan. In a laboratory setting, provided water but no food source, the maximum longevity for all parasitoids was  $\leq 3$  days.

Similar results were obtained in rearing of the Encyrtid parasitoid *Tachinaephagus zealandicus* Ashmead (De Almeida et al. 2002a), where at temperatures from 16-22°C both male and female parasitoids given food and water lived up to two times longer than those with access to water alone. Interestingly, this effect diminished with increasing temperatures at which adults

were held. *T. zealandicus* was later observed (De Almeida et al., 2002) to attack significantly more pupae when females had access to both food (provided as honey) and water than when given only water; females given neither food nor water had the lowest attack rates. Additionally, no correlation could be made between feeding treatment and fecundity, except in the instance of females given neither food nor water. Since sugar metabolism is not required for maturation of parasitoid eggs (Quicke 1997), it is logical to assume that providing most parasitoids with a sugar-based food source would not substantially affect the number, if not the quality, of progeny produced.

The number of offspring produced by many parasitoids is dependent on adult body size and food availability. It has been demonstrated (Ueno and Ueno 2007) that providing the Ichneumonid parasitoid *Itopectis naranyae* Ashmead with hosts increases their fecundity; while *I. naranyae* females could mature (chorionate) eggs without host-feeding, feeding produced an increase in future egg clutch size. Egg clutch size appears to be at least partially connected to the geographical regions in which strains develop, as is documented in the case of *Muscidifurax raptor* Girault and Sanders strains (Legner 1969; Legner 1979). *M. raptor* strains from Peru are almost completely solitary in laying of egg clutches, whereas the Chilean strain of this same insect demonstrates a higher instance of gregariousness. If tendency toward solitary oviposition is favored with smaller hosts, and gregarious oviposition more common in larger hosts (Godfray 1994), then it could be hypothesized that *T. nigra* is more likely a gregarious parasitoid.

The objectives of these experiments were twofold. The first objective, with regard to adult parasitoids, was to establish how long male and female *T. nigra* parasitoids live, respectively, when provisioned solely with water, with water and a carbohydrate food source, with water and a potential host, and with water, a carbohydrate food source plus potential hosts. The second

objective, with regard to the F<sub>1</sub> generation produced by adults in Objective (1), was to examine how the above four treatments affect female fecundity with emphasis on parasitism rate, number of progeny produced by female parasitoids, and the sex ratio of progeny produced in the F<sub>1</sub> generation.

## **Materials and Methods**

### **Study Site**

Male and female *T. nigra* specimens for the bioassay were combined from two strains reared at the USDA/ARS Center for Medical, Agricultural and Veterinary Entomology (CMAVE), Gainesville, FL. Rearing of parasitoids occurred at the CMAVE's Quarantine facility. All host flies, including *Sarcophaga bullata* Parker and *Stomoxys calcitrans* (L.), were reared in the CMAVE's colony facility. The following experiment was conducted at the CMAVE's Quarantine facility.

### **Parasitoids**

To provide adequate numbers of insects for experimentation, a Kazakh strain (KzTn) and a Russian strain (RuTn) of *T. nigra* were combined. Both strains were reared on ~2-day old pupae of *Sarcophaga bullata*. Newly emerged *T. nigra* adults were transferred to clear plastic containers (25W x 14L x 12H cm). Honey was applied to muslin strips (approx. 15 x 8 cm) and placed on the upper interior wall of the containers, and to cotton balls placed inside ~30ml plastic cups (Sweetheart Cup co., Owings Mills, MD). Water was provided *ad libitum* on cotton balls in 30ml plastic cups. Adult wasps were provisioned with ~250 cc fresh, unstung 2- to 3-day old *S. bullata* pupae. Parasitoids were exposed to the pupae for 5-6 days on a weekly basis until the death of adult wasps, to establish an F<sub>1</sub> generation, which was used in experimentation.

## **Longevity Arena**

Arenas consisted of translucent 60-ml plastic containers (Sweetheart Cup co., Owings Mills, MD) measuring 5.5 cm in height and 7 cm in diameter with matching snap-lock plastic lids. To provide air flow and exposure to honey and water, and to prevent parasitoids from escaping, a 5-cm dia. circular piece was removed from the center of each lid with a scalpel and replaced with circles of fine copper mesh (#50 size) fastened over the holes with a hot glue gun. Twenty arenas were constructed to test four treatments with five repetitions per treatment.

Food and water were provided to wasps by placing a cotton ball saturated with either a honey-water solution or water placed atop the mesh cover, allowing a portion of the mesh to remain uncovered for air flow (Figure 4-1).

## **Experimental Design**

### **Longevity determination**

The experiment was designed to assess the survivorship of male and female adult *T. nigra* when provided solely with water, with water and a carbohydrate food source (honey), with water and a host, and with honey and water as well as a host. Food consisted of a 50% w/w solution of clover honey and water kept refrigerated at 12-13°C to prevent spoilage. Water was obtained from the tap. Hosts consisted of 2-day old pupae of *S. calcitrans* (L.). Prior to exposure to *T. nigra* in arenas, *S. calcitrans* pupae were washed, dried and sorted into groups of 200 for ease of replacement on a daily basis.

Host pupae were presented to the pupae-and-water and pupae-food-and-water treatments for a period of 24 h by gently pouring 200 pupae into the rearing container. Exposed pupae were removed from the arenas at the end of each 24 h period. Adult parasitoids were manually separated from the pupae by gently shaking them through a metal sieve (#12 mesh size). Dead wasps were removed, counted and sexed. The remaining, live wasps were returned to the

appropriate arenas with 200 fresh *S. calcitrans* pupae. Ten sets of 200 pupae (five repetitions of two treatments) were recovered from the arenas after each 24 h period.

### **Fecundity estimation**

Fecundity of parasitoids with respect to treatment was determined by holding exposed *S. calcitrans* pupae from each day of the longevity experiment. The number of pupae which did not produce adult flies and the number of pupae which produced adult parasitoids were counted. Pupae collected daily from the longevity experiment were placed in ~60 ml translucent plastic cups (Sweetheart Cup co., Owings Mills, MD) with plastic lids and retained in an incubation chamber at 25 ° C until *S. calcitrans* adults emerged (emergence time was between two and four days post-wasp exposure). Adult flies were not given food or water, and died within 48 h.

Dead flies and empty puparia were removed by hand. Pupae which did not produce adult flies were placed individually into size “0” gelatin capsules, which were then retained in ~60 ml translucent plastic cups (Figure 4-2), sorted by treatment (i.e., food, water and hosts or water and hosts). Cups of encapsulated pupae were returned to the 25° C ( $\pm 0.4^{\circ}\text{C}$ , 70% RH) incubator until adult wasps emerged. The numbers of wasps that emerged per pupa per day were counted for each treatment, and total numbers of male and female progeny counted to observe the sex ratio of the F<sub>1</sub> generation.

Pupae which produced neither flies or parasitoids were considered “dud” pupae (Petersen and Meyer 1985). Ten of these pupae were dissected per treatment daily to determine whether they had been parasitized by *T. nigra* but the developing parasitoids did not survive to adult eclosion (“mummy” pupae), or if no observed parasitism occurred. The total number of mummy parasitoids for each day was estimated with the equation (Ferrero)

$$T_M = (M/10) * (T_N - P_P)$$

where

$T_M$  = total number of pupae which did not produce adult flies or parasitoids,

$M$  = total number of pupae from ten dissections which contained dead immature parasitoids,

$T_N$  = total number of pupae which did not produce adult flies, and

$P_P$  = total number of pupae which did not produce flies but did produce adult parasitoids.

The total number of parasitized *S. calcitrans* pupae for each day and treatment was calculated as

$$T_P = P_P + T_M$$

where

$T_M$  = total number of parasitized pupae which did not produce adult flies or parasitoids, and

$P_P$  = total number of pupae which did not produce flies but did produce adult parasitoids.

### **Statistical Analysis**

The adult longevity data for each treatment and for each sex were analyzed via two-way analysis of variance (Proc GLM; SAS Institute 2000) using honey, pupae, and honey \* pupae as model effects. Treatment means were separated using Tukey's means separation analysis. Unless otherwise stated, *P* values of less than 0.05 were considered statistically significant.

For fecundity data (i.e., fly pupae that were parasitized, and the resultant parasitoid progeny) statistical tests factored in sex of *T. nigra* progeny when noted; for all other progeny data analyzed, male and female numbers were combined. One-way analysis of variance (ANOVA) was performed (SAS Institute 2000) with the total number of parasitized pupae, the total number of mummy pupae, the cumulative number of male progeny, the cumulative number of female progeny, the percentage of female progeny, and the total number of parasitoid progeny, respectively, as dependent variables. This was done to determine if the water treatment only versus the water and honey treatment produced significant differences in any of the above

parameters. Data analyzed in the one-way ANOVA were subjected to arcsine transformation and analyzed as proportions.

Regression analyses were performed on the numbers of *T. nigra* progeny that emerged per pupa for both of the pupae-included treatments to determine whether a relationship existed between the presence or absence of parental feeding and resultant progeny. Regression analyses were also performed to determine whether a relationship existed between parental feeding and number of progeny (and whether *T. nigra* fecundity could be influenced by parental feeding).

## Results

### Longevity Determination

The longest lifespan for both sexes was observed in the treatment in which both honey and pupae were available to parasitoids, and the shortest lifespan for both sexes was observed in the treatment providing pupae and water but no honey. Mean longevity for females was between 11 and 13 days for the water-only treatment, the water-and-honey treatment, and for the water, honey and pupae treatment, respectively (Table 4-1). For females given only water, the lifespan was less (under ten days). Mean longevity for males (in days) was between five and ten days for all treatments (Table 4-1). Mean mortalities for all four treatments are plotted for males (Figure 4-3A) and females (Figure 4-3B)..

The provisioning of parasitoid females with honey resulted in a significant increase in longevity ( $F = 11.73$ ,  $df = 1, 367$ ;  $P = 0.007$ ) (Table 4-2). There was, however, a significant pupae-honey interaction ( $F = 5.91$ ;  $df = 1, 367$ ;  $P < 0.05$ ), which indicated that parasitoids given pupae and honey lived significantly longer than parasitoids given pupae alone (Table 4-1). Female parasitoids given pupae and water did not express a significant increase in lifespan ( $F = 1.83$ ;  $df = 1, 367$ ;  $P > 0.05$ ) over females given only water. Additionally, females with access to pupae but not honey died sooner than females which were provided only water (Table 4-1).

Results with male parasitoids were similar to those with females. Provisioning male *T. nigra* with honey significantly lengthened lifespan ( $F = 81.96$ ;  $df = 1, 386$ ;  $P < 0.0001$ ); providing males with pupae did not lengthen lifespan compared to the water-only males, however ( $F = 0.05$ ;  $df = 1, 386$ ;  $P > 0.05$ ). Overall, the effect of providing parasitoids with either honey, or honey and pupae, was stronger in males than in females, with the mean lifespan of males provided with honey and pupae nearly double that of the control males which received neither (Table 4-1). The interaction of honey and pupae on males was statistically significant, with males provided both living the longest of the four treatments (Tables 4-3).

### **Fecundity Estimation**

Because only two of the four experimental treatments in the Longevity Estimation involved pupae and therefore produced *T. nigra* progeny, only two treatments (water and pupae; water, pupae and honey) are discussed below. The mean number of pupae that were parasitized (killed) by *T. nigra* adults provisioned with water did not differ significantly from the mean number of pupae parasitized by adults provisioned with honey and water (Table 4-4). The number of mummy pupae (labeled “Pupae with dead immature wasps” in Table 4-4) produced did not differ significantly between treatments. The cumulative number of male and female progeny, respectively, did not differ between treatments, although the percentage of female parasitoids that emerged from successfully-parasitized pupae did differ significantly ( $F = 6.2$ ;  $df = 1, 8$ ;  $P < 0.05$ ), with slightly more female progeny emerging from pupae which were stung by females provisioned with honey as well as water (Table 4-5).

The cumulative number of pupae parasitized over time is presented in Figure 4-4. Females began parasitizing hosts immediately, and within 2 days had parasitized over half of the number of hosts that they would parasitize during their lives. By day 5, females in both treatment groups had parasitized over 90% of the total hosts that they would parasitize. When progeny production

is viewed in terms of successful production of adult progeny per day, a similar pattern is evident (Figure 4-6), with a maximum progeny production occurring in the first 2-3 days of adulthood. The number of adult progeny produced per parasitized host in the honey-fed group showed a noticeable decline over time, with pupae stung by young females producing about twice as many parasitoids (ca. 6 per host) as older females (Figure 4-6B) ( $R^2=0.5876$ ). In contrast, pupae stung by female parasitoids given only water showed no significant trend in parasitoid progeny produced over their reproductive lifetime (Figure 4-6A) ( $R^2=0.0175$ ).

## Discussion

### Longevity Determination

Because *T. nigra* is proovigenic, its eggs are fully chorionated upon emergence of adult female parasitoids from host puparia (Quicke 1997) and, as such, female parasitoids do not need to feed on their hosts in order to obtain amino acids for yolking (Heimpel et al. 1996; Heimpel and Rosenheim 1998). It was not expected that the presence of *S. calcitrans* pupae, in the absence of food, would significantly increase the lifespan of either female or male parasitoids. Likewise, because from a physiological perspective little incentive remains for female parasitoids to stay alive once they have utilized all eggs, it was predicted that giving *T. nigra* females access to host pupae would decrease their life expectancies compared to withholding pupae. In this study, female *T. nigra* lived approximately two days longer in the absence of pupae than when given free access to fresh pupae on a daily basis (Table 4-1), but this difference was not statistically significant.

In invertebrate models, the correlation between bearing young and life expectancy in females has been studied extensively, with data demonstrating that producing offspring has a negative impact on female longevity in the post-reproductive phase (Chapman et al. 1998). Mukhopadhyay and Tissenbaum (2006) suggest that the energy required to produce gametes and

develop offspring may be taken from energy that would otherwise be utilized to maintain somatic cells (those which will not produce gametic cells); if a tradeoff occurs when an animal attempts reproduction, a shortening of life expectancy would be, and indeed is, observed. This phenomenon may explain why male *T. nigra*, despite not producing or laying eggs, experienced a shortened lifespan when provided with pupae but not honey. If production of gametes is, for both sexes, energetically costly, then males that mated with females who had potential hosts available (as unstung pupae) would have utilized their gametes and no longer have any requirements to remain alive.

The increase in longevity for *T. nigra* females and males which were provisioned with both honey and pupae is unusual. It was hypothesized that the presence of a food source would increase the mean lifespan of all parasitoids because of an input of energy for physiological maintenance as well as providing additional energy to locate mates (male and female parasitoids) or hosts (female parasitoids only) (Wäckers 1994; Wäckers 1998). However, while the presence of honey does significantly affect male parasitoid longevity for *T. nigra*, the presence of pupae should not because male parasitoids neither feed nor oviposit inside pupae. The analysis indicated that the effect of honey on longevity was stronger when pupae were present than when they were absent. This was likely due to the continued presence of pupae allowing females to oviposit fewer eggs each day over a longer period of time, and therefore be more selective in their choice of host pupae.

When provided with pupae and water but no honey, both male and female parasitoids did not demonstrate a significant difference in longevity compared to conspecifics that were only given water (Table 4-1). It was not expected that providing hosts to parasitoids in the absence of a food source would extend lifespan for males. Because most female parasitoids control the

timing of fertilization of eggs and oviposition (Quicke 1997), there is little pressure on males to mate with females as quickly as possible. It was predicted that providing pupae to female *T. nigra* adults in the absence of a carbohydrate food source such as honey would decrease their lifespan due to a greater pressure on females to oviposit before depleting the energy allocated for host seeking and oviposition; however, the greater mean lifespan of females provided with water alone was not significantly different from the mean lifespan of those females provided with water and pupae (Table 4-1). Indeed, this indicates that, as with males, the life-lengthening effects of honey were greater when host pupae were present than when they were absent. This may be because the energetic demands required for drilling and oviposition by female *T. nigra* were lessened with the availability of energy via honey.

### **Fecundity Estimation**

Of the parameters analyzed via two-way ANOVA (Table 4-4), only the cumulative numbers of killed pupae differed significantly between treatments (pupae only, versus pupae and honey). The increase in number of pupae killed by female parasitoids given both honey and pupae ( $856.8 \pm 30.3$ ) compared to females provided pupae and no honey is because a greater number of pupae were killed between d 4 and 9 (Figure 4-4). Both treatments demonstrated greatly reduced killing of host pupae by d 11, with the majority (>90%) of cumulative pupae killed by d 10. Since many parasitoids have demonstrated a higher fecundity rate and produce longer-lived progeny when provided with older hosts (de Almeida et al. 2002; Bellows, Jr. 1985), the influence of host age on data collected from parasitoid progeny in this experiment was controlled for by utilizing host pupae of a strictly defined age.

On average, each female of *T. nigra* produced about 23 adult progeny over her lifetime. This is substantially higher than the 10.5 and 3.6 progeny produced per female of *T. stomoxydis* (Morgan et al.. 1990) and *T. painteri* (Huggert and Morgan 1993), respectively. It is similar to

the lifelong fecundity figure of 21.4 progeny per female of the encyrtid larval parasitoid *Tachinaephagus zealandicus* Ashmead when *T. zealandicus* is supplied with house fly pupae (Geden et al., 2003). Most of the research on filth fly parasitoids has concentrated on pupal ectoparasitoids in the family Pteromalidae. *Spalangia endius* Walker, which has been used successfully as a fly biological control agent, produces only about half as many progeny per female as *T. nigra* (Morgan et al., 1976). *Spalangia cameroni* Perkins, another commonly used fly parasitoid, has a longer lifespan than *S. endius* and produces about 30-40 progeny per female (Legner and Gerling 1967, Moon et al. 1982).

Perhaps the most widely used parasitoid for fly control are *Muscidifurax raptor* and related species in the same genus. Lifelong fecundity estimates for *M. raptor* vary from 26-185 progeny per female over her lifetime depending on the strain and health of the colonies under consideration (Morgan et al., 1979, Zchori-Fein et al. 1992). Fecundity of *T. nigra* therefore is squarely within the range of several other economically important species of muscoid fly parasitoids. The geographic distribution of *T. nigra* (Geden and Moon, unpublished data) across eastern Europe is also within the distribution of other common filth fly parasitoids, such as *S. endius* Walker, *S. cameroni* Perkins, and *M. raptor* (Hogsette et al. 1994, 2000). Having identical host milieus and overlapping geographical distributions indicate that in the wild, these species likely inhabit complementary niches. It would be interesting to determine in the future whether introduction of *T. nigra* in conjunction with other commercially-important parasitoids of filth flies (e.g., *S. endius*, *S. cameroni*, and *M. raptor*) increases control of flies that both species are known to parasitize, such as *M. domestica* and *S. calcitrans*.

Future experimentation involving the life history of *Trichopria nigra* will utilize a larger sample size for longevity studies. It would also be prudent to feed adult *T. nigra*, particularly

females, on a variety of sugar-based food sources (such as plant nectar, honey, glucose solution), to more accurately determine whether an artificial (laboratory) diet significantly extends or shortens the lifespan of this parasitoid, compared to the lifespan when fed on a diet more accurately mimicking their natural diet. Additionally, it is not known at this time whether feeding females before allowing them access to host pupae, rather than providing both at the same time, affects the number of progeny that are produced successfully. In this experiment, *T. nigra* in the water, honey and pupae treatment were given access to all three factors from day 0; perhaps withholding pupae and feeding parasitoids first would incur a benefit of some sort.

Table 4-1. Mean lifespans ( $\pm$ SE) (d) of *Trichopria nigra* adults under different feeding treatments at 25 °C.

Treatment	♀ <i>n</i>	♀ Mean (SE) lifespan (d)	♂ <i>n</i>	♂ Mean (SE) lifespan (d)
Water only	90	11.94 ( $\pm$ 0.51)ab	102	6.71 ( $\pm$ 0.33)b
Water + honey	102	12.53 ( $\pm$ 0.52)a	97	8.97 ( $\pm$ 0.35)a
Water + pupae	80	9.85 ( $\pm$ 0.52)b	94	5.46 ( $\pm$ 0.30)b
Water + honey + pupae	99	12.99 ( $\pm$ 0.52)a	97	10.05 ( $\pm$ 0.49)a

Means in the same column followed by the same letter are not significantly different according to Tukey's means separation analysis (SAS 2000).

For females,  $F=6.49$ ;  $df=3, 367$ ;  $P<0.001$ . For males,  $F=30.54$ ;  $df=3, 386$ ;  $P<0.001$ .

Table 4-2. Female *T. nigra*, two-way ANOVA results for influence of presence/absence of honey and host pupae on survival time. Numbers marked with an asterisk are considered significant ( $P < 0.05$ ). Water alone was considered the control, therefore water is not included as a model effect.

Model effect	df	Sum of Squares	Mean Square	<i>F</i>	<i>P</i>
Honey	1	297.5	297.5	11.73	0.0007*
Hosts	1	46.4	46.4	1.83	0.1769
Honey x Hosts	1	150.0	150.0	5.91	0.0155*
Error	367	9309.3	25.4		

Table 4-3. Two-way ANOVA results for influence of presence/absence of honey and host pupae on survival time of male *T. nigra*. Water alone was considered the control, therefore water is not included as a model effect.

Model effect	df	Sum of Squares	Mean Square	<i>F</i>	<i>P</i>
Honey	1	1129.2	1129.2	81.96	<0.0001*
Hosts	1	0.8	0.8	0.05	0.8151
Honey x Hosts	1	132.3	132.0	9.60	0.0021*
Error	386	5318.2	13.8		

Numbers marked with an asterisk are considered significant ( $P < 0.05$ ).

Table 4-4. Effect of diet on mean number ( $\pm$ SE) of pupae killed by female *T. nigra*, pupae producing wasps, having dead wasps, and total number of parasitized pupae.

Treatment	Number of Pupae Killed	Number of Pupae Producing Wasps	Number of Pupae with dead immature wasps	Cumulative Number of Pupae Parasitized
Pupae Only	715.4 ( $\pm$ 48.1)	47.0 ( $\pm$ 6.0)	163.6 ( $\pm$ 19.9)	210.6 ( $\pm$ 15.5)
Honey and Pupae	856.8 ( $\pm$ 30.3)	46.4 ( $\pm$ 7.5)	172.1 ( $\pm$ 21.4)	218.5 ( $\pm$ 27.7)
ANOVA F	6.2*	<0.1ns	0.09ns	

df = 1, 8;  $P < 0.05$

Table 4-5. Cumulative ( $\pm$ SE) numbers of male and female progeny, respectively, produced by parasitoids given only water and pupae, or water, honey and pupae.

Treatment	Cumulative Number of Male Progeny	Cumulative Number of Female Progeny	Cumulative Number Progeny Produced	Percent Female
Hosts Only	141.8 ( $\pm$ 42.9)	83.3 ( $\pm$ 22.1)	225.0 ( $\pm$ 27.2)	37.0 ( $\pm$ 1.9)
Honey and Pupae	229.4 ( $\pm$ 41.3)	107.8 ( $\pm$ 43.3)	237.2 ( $\pm$ 36.7)	45.4 ( $\pm$ 2.6)

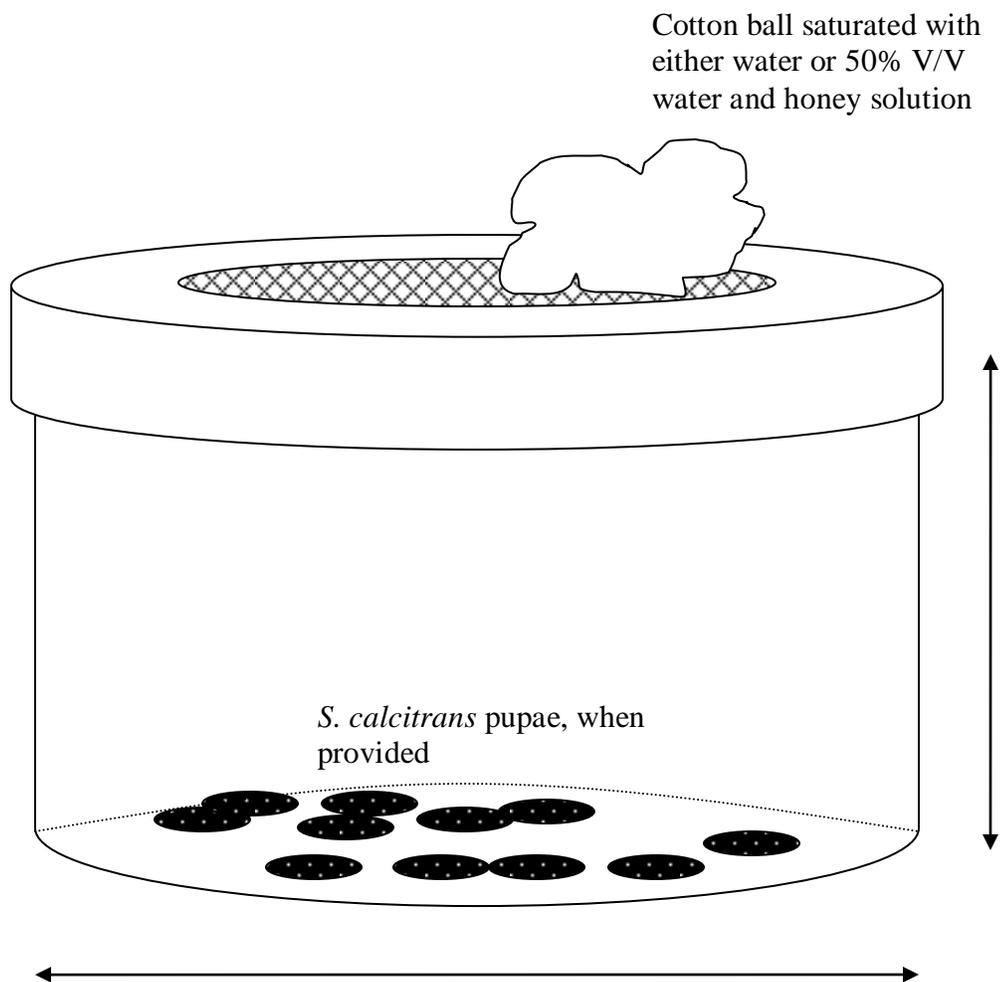


Figure 4-1. Rearing containers for longevity determination. Air flow and access to water and/or food was provided via mesh screen on container lids. Five repetitions were provided with water, five with water and 200 *S. calcitrans* pupae, five with a 50% V/V water and honey solution, and five with a 50% V/V water and honey solution and 200 *S. calcitrans* pupae.

A)



B)



Figure 4-2. 60-ml cups containing individually-encapsulated *S. calcitrans* pupae. A) Following emergence of flies from unparasitized pupae, those pupae which did not produce flies were each placed into a gelatin capsule so that the number of wasps emerged from each successfully-parasitized pupae could be observed. B) Successfully-parasitized pupa.

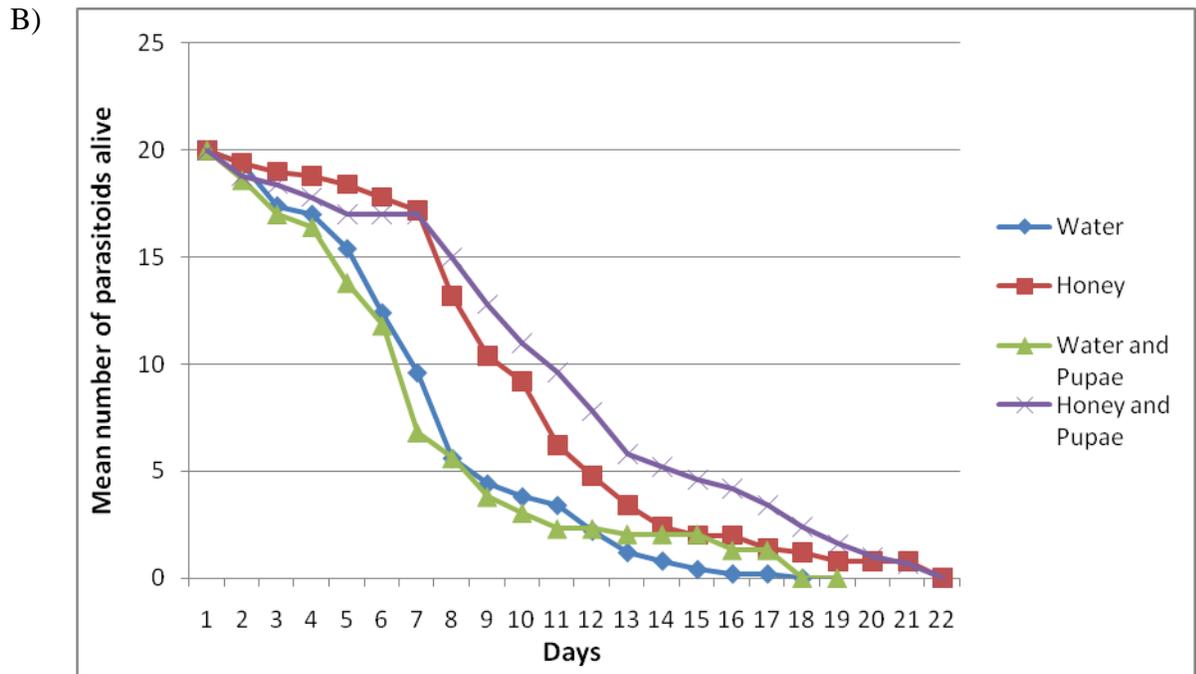
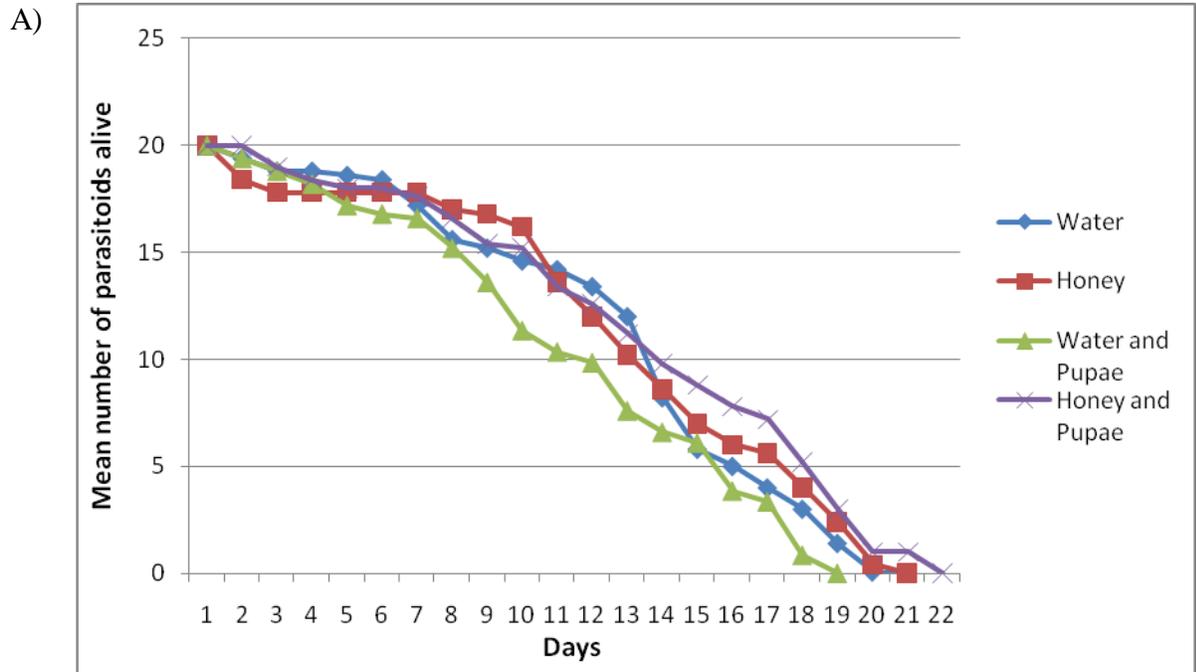


Figure 4-3. Mean adult A) female and B) male *Trichopria nigra* mortality for all treatments.

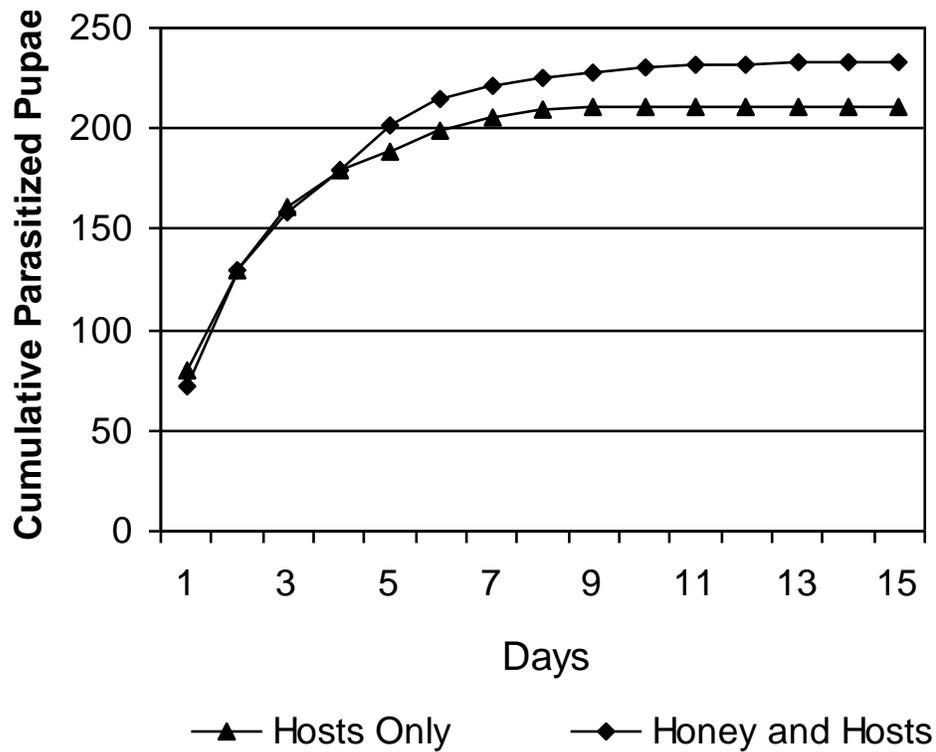


Figure 4-4. Total number of *S. calcitrans* pupae parasitized by female parasitoids for each of two treatments (water and hosts, food and hosts, respectively). The total number of parasitized pupae was defined as the sum of pupae which successfully produced adult parasitoids and pupae which contained immature parasitoids that never emerged. After days 9-10, no adult parasitoids emerged from pupae.

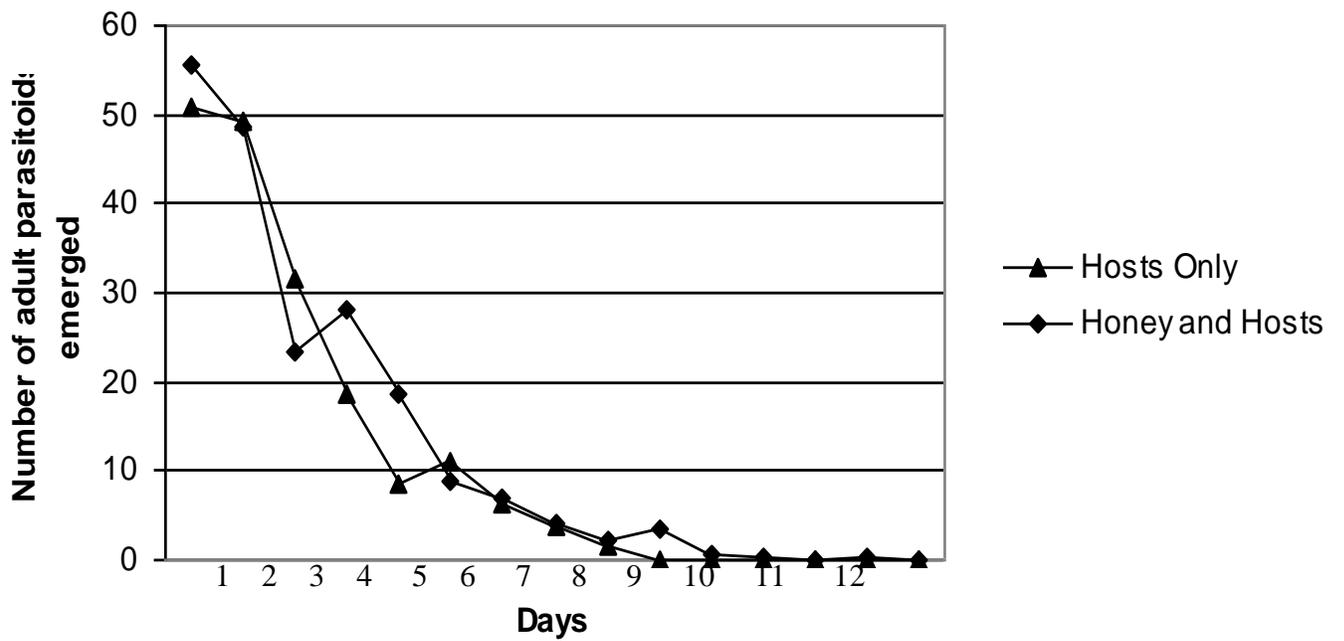
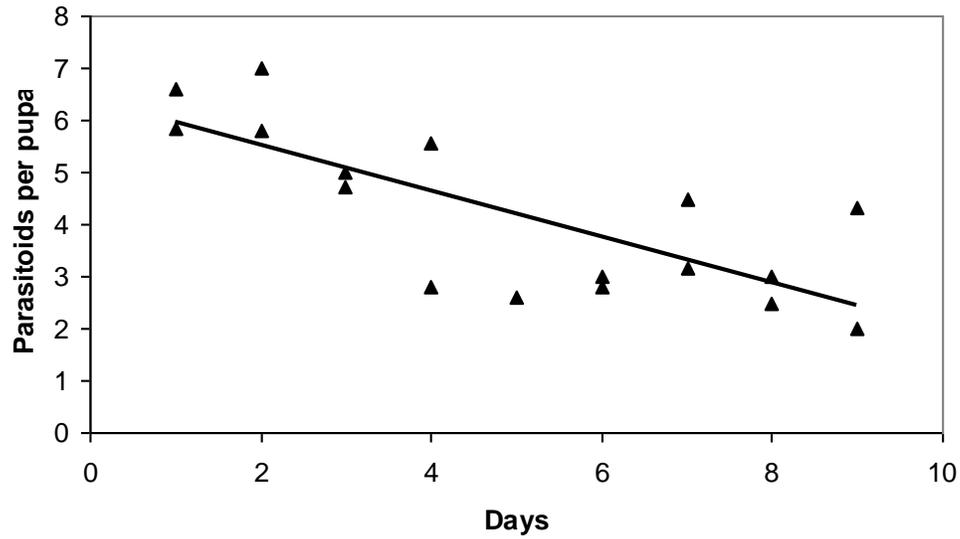


Figure 4-5. Mean number of adult parasitoids that emerged daily for each of two treatments (water and hosts, food and hosts, respectively). After days 6-7, very few parasitoids emerged. The highest numbers of adult emergence were observed from day 1 through day 3.

A



B

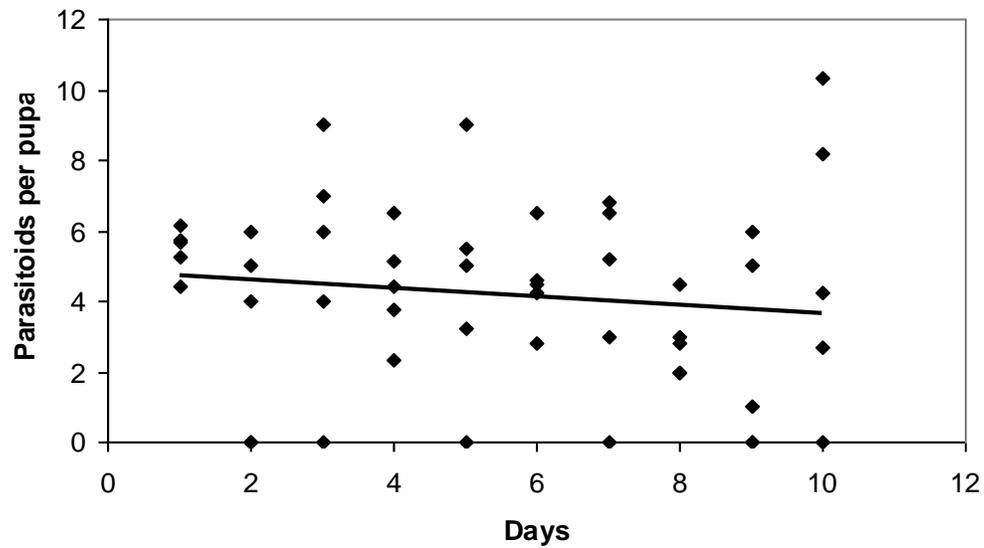


Figure 4-6. The average number of parasitoids that emerged per pupa per day. A) Water and hosts only. B) Food, water and hosts. The average number of parasitoids per pupa was calculated for each replicate, and averages plotted. The  $R^2$  value for the regression line in A) = 0.5876. The  $R^2$  value for the regression line in B) = 0.0175.

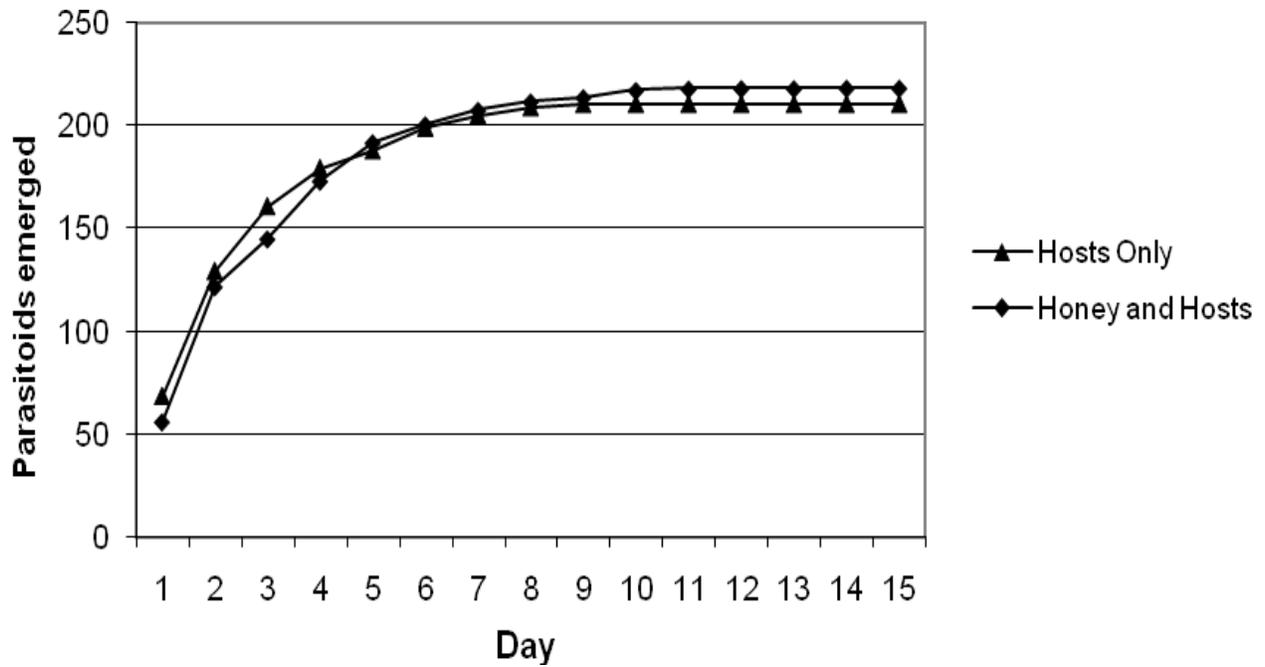


Figure 4-7. Cumulative mean number of adult parasitoids that emerged from *S. calcitrans* pupae for each of two treatments (water and hosts, food and hosts, respectively). After days 6-7, very few adult parasitoids emerged.

## CHAPTER 5 ARENA-CHOICE EXPERIMENTS

### **Introduction**

Host specificity is a common feature found in many parasitoid genera. In general, koinobionts (insects which parasitize a host in the early stages of host development, and in which the host continues to develop) demonstrate a higher level of host specificity than do idiobionts (Althoff 2003); this aspect of parasitoid evolution is most likely due to the necessity of a koinobiont parasitoid being able to develop and mature in confluence with a host with a continuously changing endocrine milieu. Idiobionts, by contrast, parasitize their hosts in later stages of development and, because the host insect may go through few if any endocrinological changes such as change of instar that influence parasitoid development, the host is often “arrested” in development and does not survive long (Quicke 1997). Because the nutritional and physiological dependence of koinobiont parasitoids on their hosts is greater compared to externally-living idiobiont parasitoids, host ranges of koinobionts tend to be narrower than those of idiobionts (Hochberg and Ives 2000; Godfray 1994).

Parasitoid species possess specific and innate ranges of hosts they can successfully parasitize. Askew and Shaw (1986) hypothesized that koinobiont parasitoids would be more host-specific than idiobionts because of the internal development and nutritional and endocrinological dependencies that koinobionts have on their hosts. Other *Trichopria* species have been found to parasitize *Stomoxys calcitrans* (L); *T. stomoxydis* Huggert and *T. painteri* Huggert and Morgan are known only to parasitize that species (Morgan et al. 1990; Huggert and Morgan 1993). Another closely related species, *T. anastrephae* Lima, was found to parasitize pupae of *Anastrepha* species in western Brazil (Garcia and Corseuil 2004).

The question of whether parasitoids are innately competent to locate hosts, or whether prior exposure to a host (or a host's surroundings) improves chances of locating a host and laying eggs, is an important one. Little is known about the effects of conditioning on fly pupal parasitoids, and even less about conditioning on parasitoid species in the family Diapriidae, such as *T. nigra* (Nees). More generally, it is known that for many parasitoids, associative learning is an important aspect of host location and selection. Hopkins' Host-Selection Principle has been observed in many insects (Craighead 1921; Smith and Cornell 1979; Davis and Stamps 2004). According to this principle, first observed in herbivorous insects (Craighead 1921), the host an insect develops within provides the first visual and olfactory conditioning, so that adult insects can later locate the same host they developed within, for laying their eggs.

The odors and visual presence associated with pupae often serve as stimuli (see Jandt and Jeanne 2004 for example) that parasitoids associate with a reward (i.e., oviposition, or feeding prior to oviposition). Odor stimuli can be either compounds from either potential host pupae or their surrounding environment (Sullivan et al. 2000; de Jong and Kaiser 1992). In some instances, host location stimuli are visual rather than olfactory, as is the case for the phorid fly parasitoid of fire ants, *Apocephalus paraponera* Borgmeier (Morehead and Feener 2000). Regardless of the type of stimulus, learning is an adaptive behavior. Once conditioned to seek out olfactory or visual cues, the foraging time for future oviposition events is often shortened, maximizing the number of hosts parasitized and eggs laid by the parasitoid (Stireman III 2002).

*Diadromus pulchellus* Wesmael (Hymenoptera: Ichneumonidae), a parasitoid wasp which attacks larvae of the moth *Acrolepiopsis assectella* Zeller, appears to utilize volatile compounds emitted from *A. assectella* larvae and their frass to locate possible hosts for oviposition (Lecomte and Thibout 1993). When adult female parasitoids are exposed to either frass or larval

hosts prior to placement in an olfactometer, the number of turns made before choosing the host/frass odor over a control was fewer than those made by females never exposed to hosts or frass post-emergence (Lecomte and Thibout 1993). However, when naïve *D. pulchellus* females were exposed to volatiles from *A. assectella* larvae and a control in an olfactometer, they did not show any significant differences in behavior when compared to female *D. pulchellus* reared on an atypical host, *Plodia interpunctella* (Hubner). These findings indicate that, for some species, post-developmental exposure to a host is a critical step for females to learn host selection.

In the laboratory, *Trichopria nigra* (Nees) successfully parasitizes pupae of a number of nuisance flies, including *Stomoxys calcitrans* L., *Ophyra aenescens* (Wiedemann), *Haemotobia irritans* (L.), and *Sarcophaga bullata* (Parker) in the laboratory (Geden and Ferrero, personal observation). Additionally, colonies of *T. nigra* have been successfully maintained utilizing these same fly species as hosts. *T. nigra* readily probes pupae of *Musca domestica* and appears to oviposit; although pupal mortality in the host is high, no adult parasitoids have emerged in numerous laboratory rearing attempts (Geden, unpublished data). Other dipteran hosts parasitized by *T. nigra* include *Piophilidae casei* (L.) (Teodorescu and Ursu 1979) and tachinid flies (Nash 2005).

*T. nigra* demonstrates an unusual life history, with respect to the interplay of physiology and behavior of parasitic wasps. Unlike many other endoparasitoids, it develops inside a pupal host rather than a larval one. Furthermore, the ability of *T. nigra* to develop successfully in a number of hosts is atypical of endoparasitoids. Conditioning studies involving *T. nigra* cannot be found in the literature and the effects of conditioning on this parasitoid are unknown. Therefore, the first objective of these experiments was to determine if conditioning *T. nigra* to a particular host via exposure would bias *T. nigra* females to choose their “conditioning type” host when

presented with multiple host choices. The second objective was to determine if rearing *T. nigra* on two different host species affects their likelihood of choosing the same species, when newly-emerged F<sub>1</sub> parasitoids are presented with a choice of multiple hosts.

## **Materials and Methods**

### **Study Site**

Parasitoids and host flies were reared at the USDA/ARS Center for Medical, Agricultural and Veterinary Entomology (CMAVE), Gainesville, FL. The following experiment was conducted at the CMAVE's Quarantine facility.

### **Hosts**

The host fly species *Stomoxys calcitrans* (L.), *Musca domestica* L. and *Sarcophaga bullata* Parker were taken from USDA colonies. Pupae utilized for both experiments were collected from rearing containers 2-3 days following pupation and either used immediately, or stored in chambers at 14°C (70% RH) for up to four days to retard physiological development and emergence of adult flies. It has been demonstrated that holding pupae of these species at similar temperatures (see Moribayashi et al. 1999, Leopold et al. 1997) does not significantly affect quality or survivorship of the insect.

### **Parasitoid strains**

The *T. nigra* adults used for the bioassay were combined from two strains reared separately and were later pooled to provide adequate numbers of parasitoids. A Kazakhi strain and a Russian strain, both maintained on *S. bullata* pupae and assumed to be identical in physiology and behavior, were used for experimentation. For details on rearing of *T. nigra*, see Chapter 3.

### **Conditioning parasitoids for short-term assay**

Three days after emergence from *S. bullata* pupae [to provide adult wasps with adequate time for wing and exoskeleton hardening, maturation of ovarioles, and opportunities to mate]

male and female *T. nigra* were removed from their rearing containers with an aspirator. The wasps were combined then divided into fourths and placed into 250 ml paper cups containing 140 cm<sup>3</sup> of either *S. bullata*, *S. calcitrans*, or *M. domestica* pupae, or no pupae (control) for the purpose of conditioning wasps to pupae of one species.

The 250 ml paper cups containing wasps and treatments were secured across their openings with cotton cloth to allow air flow and prevent wasp escape. Atop each of the four cotton cloth covers was placed a cotton ball saturated with distilled water, and a streak of honey was applied to the cotton to provision wasps with food. Cups were then placed in an incubator (25 C ±0.4 C, 70% RH) for 48 h, after which the wasps were separated from the pupae by placing both in a #12 mesh screen sieve and shaking gently so *T. nigra* adults would fall onto a clean piece of white paper for collection. Wasps were then immediately introduced to arenas for experimentation.

#### **Arena for short-term assay**

Arenas consisted of clear plastic containers measuring ca. 24 x 24 x 10 cm (L x W x H) with plastic airtight snap-closure tops (Glad Products company, Oakland CA). Into each arena were placed four ~5 cm-dia. plastic Petri dish bottoms each containing 2.0 g. *S. bullata* pupae, 2.0 g. *S. calcitrans* pupae, 2.0 g. *M. domestica* pupae, or an empty (blank) Petri plate serving as a control (Figure 5-1). Petri plates were arranged in a randomized block design between repetitions. Five repetitions were conducted for each of four treatments, and the assay was repeated twice for a total of ten repetitions per treatment. Arrangement of Petri plates was to provide maximum distance between choices (approx. 8 cm between plates).

The wasps which had been conditioned to different fly species were introduced to arenas by aspirating them following sieving procedure, and then gently blowing a pea-sized ball of parasitoids into the center of each arena. Plastic lids were immediately secured tightly on

containers to prevent wasp escape during the assay. Containers were left for 2 hours to provide wasps with adequate time to choose a host species and initiate probing and/or oviposition. At 2 h, plastic lids were removed from the arenas, covers were placed on Petri plates to contain wasps, and the Petri plates were frozen for 12h to kill wasps. Dead male and female wasps in each of the Petri plates were counted.

### **Parasitoids for rearing assay**

Adult (5-day old) *T. nigra* from the combined strains were divided into two plastic rearing containers (25 cm L x 14 cm W x 12 cm H, see Chapter 3 for further details on rearing methods). One container of wasps was provisioned with 150 cm<sup>3</sup> of *S. bullata* pupae, and the other with 150 cm<sup>3</sup> of 2-3-d old *S. calcitrans* pupae, in 250 ml paper cups. The layer of pupae was approximately 3-5 cm deep. The cups of pupae were held inside their respective *T. nigra* rearing containers for 6 d to allow time for females to oviposit in the puparia.

After 6 d, the paper food cups of pupae were removed from the *T. nigra* rearing containers, adult parasitoids shaken off with a #12 mesh sieve and discarded, and cloth covers secured across the opening of the containers (now containing only potentially-stung pupae). The containers were placed in an incubator (25° ±0.4°C, 70% RH) and held until the emergence of the next generation of parasitoids. Upon emergence of parasitoids, a cotton ball saturated with distilled water and a streak of honey was placed atop the cloth cover to provide parasitoids with moisture and a food source. Wasps were introduced to the experimental arena following a 3-d maturation period.

### **Arena for rearing assay**

Arenas consisted of clear plastic containers measuring 24 x 24 x 10 cm (L x W x H) with plastic airtight snap-closure tops (Glad Products company, Oakland CA), of similar design to those in the short-term host exposure assay. Into each arena were placed three ~5-cm dia. plastic

Petri dish bottoms each containing 2.0 g. of *S. bullata* pupae, 2.0 g. *S. calcitrans* pupae, or an empty (blank) Petri plate serving as a control (Figure 5-2). Petri plates were placed in a randomized block design between repetitions. Five repetitions were conducted for each of two treatments. Petri plates were arranged to provide maximum distance between choices (approx. 10 cm between plates).

The wasps reared on either *S. bullata* pupae or *S. calcitrans* pupae were introduced in pea-sized balls to arenas via a manual aspirator, one group for each arena. Plastic lids were immediately secured tightly on containers to prevent wasp escape during the assay. Containers were left for 2 h as in the previous test. At 2 h, plastic lids were removed from the arenas, covers were placed on Petri plates to contain wasps, and all arenas were frozen for 12 h. Dead male and female wasps in each of the Petri plates were counted.

### **Statistical Analysis**

For the short-term host exposure assay, numbers of wasps in each Petri plate were separated and analyzed by sex. The numbers for the five repetitions were averaged for each treatment and the mean raw numbers of male and female wasps exposed to each treatment compared using Tukey's HSD test ( $P=0.05$ ; SAS Institute 2000). Conditioned wasps were compared with unconditioned parasitoids for host preference via one-way analysis of variance (SAS Institute 2000). Additionally, an arcsin transformation was performed on mean numbers of wasps to correct for significant variation in total numbers of wasps among repetitions, and the proportions analyzed using Tukey's HSD test ( $P=0.05$ ; SAS Institute 2000).

For the rearing assay, numbers of wasps in each Petri plate were separated and analyzed by sex. Following sexing of parasitoids in each of the Petri plates, numbers for repetitions were averaged for each treatment and the mean numbers of male and female wasps exposed to each treatment were compared using Tukey's HSD test ( $P=0.05$ ; SAS Institute 2000). Additionally, an

arcsin transformation was performed on mean numbers of wasps to correct for significant variation in total numbers of wasps among repetitions, and the proportions analyzed using Tukey's HSD test ( $P=0.05$ ; SAS Institute 2000).

## Results

No significant differences were observed between the proportions of female parasitoids conditioned on *S. calcitrans* pupae which were counted in (e.g., assumed to have oviposited in) Petri plates containing different pupal types (Table 5-1). The Petri plate of *M. domestica* pupae lured a significantly greater proportion of female parasitoids ( $df=1$ ;  $P<0.0001$ ) than the pupae of the other two species (Table 5-1). Although numerically more female parasitoids which were conditioned with *S. bullata* pupae went to that species' pupae than did female parasitoids conditioned to other hosts, the difference was not significant ( $0.051$ ). Male parasitoids conditioned to *S. bullata* pupae demonstrated no significant preference to any of the Petri plates of pupae, regardless of conditioning (Table 5-2). Unconditioned parasitoids of either sex did not demonstrate any significant preference for a particular host fly species' pupae.

The host in which the female parasitoids were reared had a significant impact on their behavior in the experiment ( $df=1$ ;  $P<0.0001$ ), with approximately twice as many females choosing their natal host rather than a non-natal host (Table 5-3). This preference for natal host was not observed in males ( $df=1$ ;  $P=0.57$ ) reared on either *S. calcitrans* or *S. bullata* pupae (Table 5-4).

For the host-exposure experiment, post-experiment counts (total number of wasps were counted for five of the twenty containers) determined that the groups introduced to the arenas averaged  $589 \pm 16$  wasps of mixed sex per arena, equating to a "pea-sized ball". For the rearing experiment, , post-experiment counts (total number of wasps were counted for five of the twenty

containers) determined that the groups introduced to the arenas averaged  $432 \pm 21$  wasps of mixed sex per arena.

### **Discussion**

The large proportion of female *T. nigra* responding to the visual and olfactory cues provided in the experimental arena, as well as females observed and counted while attempting to oviposit on all fly species' pupae presented in the experimental arenas suggest that in the wild *T. nigra* may attempt to oviposit inside any of these species and use them as hosts. The results of these experiments indicate that the host range of *T. nigra* is fairly broad, especially for a koinobiont (Whitfield 1998; Quicke 1997). Additionally, other species of *Trichopria* have been shown to have wide host ranges (Morgan et al. 1990; Garcia and Coseuil 2004). Such an assumption must be made with caution, however, as it has been demonstrated that hymenopterous insects can be readily conditioned to artificial stimuli (Jong and Kaiser 1991; Jandt and Jeanne 2004).

Another reason for caution is the role of host habitats in directing searching behavior in *T. nigra*. Parasitoids typically follow a series of steps that begin with host habitat location (Vinson 1976). If *T. nigra* is conditioned to search for carrion rather than dung, then carrion-inhabiting flies, e.g., calliphorids and sarcophagids, should be parasitized by *T. nigra* more often than stable flies. Field research in the home range of this species is needed to determine whether parasitism of stable flies is common, or the result of chance encounters. The experiment conducted utilizing parasitoids which had successfully emerged from both *S. bullata* and *S. calcitrans* pupae, indicates that this species utilizes both host species where they both occur in the wild. Moreover, laboratory studies can sometimes suggest stronger host preferences than are evident in the field, where parasitoids are subject to more complex sets of stimuli (Mandeville and Mullens 1990a, b).

Learning among parasitoid hymenoptera is well-documented, and follows the general pattern of insect reward-driven association. Females of the fruit fly larval parasitoid *Leptopilina boulandi* Barbotin et al. (Hymenoptera: Eucoilidae), for example, were exposed to an artificial odor (i.e., one not naturally produced by the host or plants fed on by the host) in conjunction with an oviposition experience in a laboratory (de Jong and Kaiser 1992). The exposed females would demonstrate a much stronger preference for the scent they were exposed to during stimulus/reward conditioning compared to naïve female parasitoids. Conditioned females also demonstrated a much stronger preference than did their control counterparts, which were not classically conditioned to any stimulus.

Selection of a suitable host at close range is essential, and the availability of visual or olfactory cues may greatly increase a parasitoid's likelihood of encountering a potential host if that parasitoid has learned that certain cues signal host availability (Hochberg and Ives 2000). Most parasitoids whose host range is greater than one host species will often choose the most abundant host for the greatest number of oviposition events (Hastings and Godfray 1999); while possibly the result of a female parasitoid accidentally encountering the most abundant host species at a greater statistical rate, may also be explained by a greater number of visual or olfactory cues presented by the most abundant host. The number of viable host pupae available is often an indicator of host fecundity (Hochberg and Ives 2000), and is perhaps, as well, an indicator of host attractiveness to parasitoids. To this end, an abundance of all potential host species was presented in the experiments, to prevent one potential host being more available than the others.

The stronger response of female *T. nigra* tested in the rearing assay compared to those conditioned for 48 h indicate that rearing of this wasp on a particular host increases the fidelity

of adult females to forage for and oviposit in the same host as their natal species. Hopkins' Host Selection principle has often been cited as a strong motivator for insects to search for and utilize hosts which resemble their own natal hosts (Michaud and Grant 2005), although the life stage at which this induction occurs remains somewhat controversial (Barron 2001; Davis and Stamps 2004). It would seem logical that, for parasitoids with more than one potential host, initial exposure to host kairomones predisposes female parasitoids to forage for that particular host. This preference for natal host appears to be established when newly-emerged parasitoids exit their host puparia and encounter the odors associated with that species and location (Vet 1983; Vet and Groenewald 1990). Such a preference would be particularly adaptive in relatively constant habitats that support sequential generations of hosts (Vet 1983; Turlings et al. 1992).

Although the literature on insect learning is voluminous, little is known about conditioning and host selection in parasitoids of muscoid flies, and even less is known about host selection behavior in the family Diapriidae. *Nasonia vitripennis* (Walker) shows a moderate preference for the host species on which is reared, but it is not clear whether this preference represents pre-imaginal conditioning or an induction event in which newly emerged adults experience host remains and empty puparia at the time of eclosion (Oghushi 1960, Smith and Cornell 1979). In contrast, there is little evidence for rearing-host effects on host preference in parasitoids in the genera *Muscidifurax*, *Dirhinus*, or *Spalangia* (Mandeville and Mullens 1990b, Oghushi 1960). The only known report of adult conditioning of fly parasitoids is that of Mandeville and Mullens (1990b). In this study, the strong innate preference of *Muscidifurax zaraptor* for house fly over *Fannia canicularis* (L.) pupae was shifted in favor of the latter species by 2 days of experience ovipositing on that host. This shift occurred in spite of the fact that *M. zaraptor* is substantially more successful on *M. domestica* than on *F. canicularis* hosts

(Mandeville and Mullens 1990b). The observation that *T. nigra* can be conditioned to favor a host on which it can not develop (house fly [Geden and Moon 2008]) is a curious parallel to the host shift seen in *M. zaraptor*.

Due to the inability to rear *T. nigra* on pupae of *M. domestica*, choices for the rearing assay were limited to *S. bullata* and *S. calcitrans* pupae (see Chapter 2). The host-selection behavior of *T. nigra* when presented with multiple potential host insects, and the shift in that behavior (in most instances) to preferentially seek pupae of the host previously conditioned to is consistent with the current literature on associative learning in other parasitic Hymenoptera. While Hopkin's host selection principle may not be strictly valid in explaining the stronger response of parasitoids reared on multiple host species (as opposed to a brief post-emergent exposure to a particular host), certainly initial exposure of female parasitoids to host kairomones presents a case for *de facto* conditioning to a parasitoid's natal host that, in the absence of later associative learning that exposes parasitoids to different host species, establishes a default mechanism for seeking out hosts that are native to a certain geographical area. Further research should be performed to examine the means by which *T. nigra* might learn to seek out a particular host, and establish the strength of conditioning's effect on host-seeking behavior. Further research conducted involving host choice with *T. nigra* should utilize a much higher sample size, especially with regards to the rearing assay.

Table 5-1. Proportion of female *T. nigra* responding to pupae of 3 host species after prior conditioning for 48 h on pupae of a single host species. Means in rows followed by different letters are significantly different according to Tukey's HSD-test (P=0.05; SAS Institute 2000).

Conditioning Host	Mean (SE) proportion of parasitoids recovered on host			ANOVA <i>F</i>	<i>P</i>
	<i>S. calcitrans</i> pupae	<i>M. domestica</i> pupae	<i>S. bullata</i> pupae		
<i>S. calcitrans</i>	0.34(±0.10)a	0.36(±0.12)a	0.30(±0.05)a	0.81	0.50
<i>M. domestica</i>	0.27(±0.07)a	0.43(±0.10)b	0.30(±0.10)a	9.81	0.0002
<i>S. bullata</i>	0.29(±0.07)a	0.31(±0.09)a	0.40(±0.10)a	2.95	0.0512
No conditioning	0.32(±0.07)a	0.35(±0.13)a	0.34(±0.07)a	0.80	0.50

Table 5-2. Proportion of male *T. nigra* responding to pupae of 3 host species after prior conditioning for 48 h on pupae of a single host species. Means in rows followed by different letters are significantly different according to Tukey's HSD-test (P=0.05; SAS Institute 2000).

Conditioning Host	Mean (SE) proportion of parasitoids recovered on host			ANOVA <i>F</i>	<i>P</i>
	<i>S. calcitrans</i> pupae	<i>M. domestica</i> pupae	<i>S. bullata</i> pupae		
<i>S. calcitrans</i>	0.45(±0.05)a	0.23(±0.04)b	0.34(±0.04)ab	6.36	0.0056
<i>M. domestica</i>	0.40(±0.03)a	0.31(±0.02)a	0.29(±0.03) a	2.83	0.0771
<i>S. bullata</i>	0.38(±0.04)a	0.31(±0.05)a	0.30(±0.03)a	0.78	0.4697
No conditioning	0.37(±0.04)a	0.35(±0.05)a	0.29(±0.02)a	1.07	0.3593

Table 5-3. Mean proportions of female parasitoids reared on either *S. calcitrans* or *S. bullata* pupae, who selected either *S. calcitrans* or *S. bullata* pupae as a first host choice. Means followed by the same letter are not statistically significant by PROC GLM ( $P=0.05$ ).

Emerged from	On <i>S. calcitrans</i> pupae	On <i>S. bullata</i> pupae
<i>S. calcitrans</i>	0.70a	0.30b
<i>S. bullata</i>	0.36b	0.64a

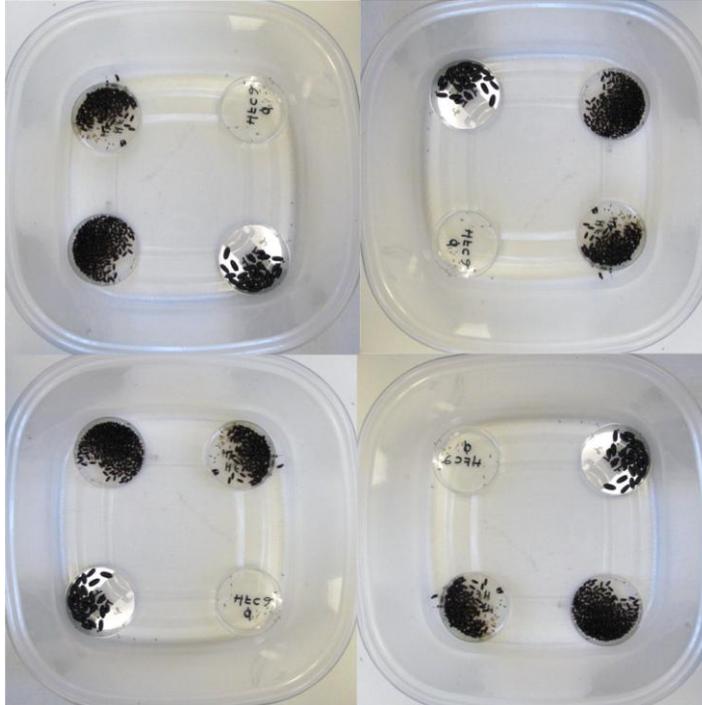
Df=1, 8;  $F= 33.25$ ;  $P < 0.0001$

Table 5-4. Mean proportions of male parasitoids reared on either *S. calcitrans* or *S. bullata* pupae, who selected either *S. calcitrans* or *S. bullata* pupae as a first host choice. Means followed by the same letter are not statistically significant by PROC GLM ( $P=0.05$ ).

Emerged from	On <i>S. calcitrans</i> pupae	On <i>S. bullata</i> pupae
<i>S. calcitrans</i>	0.45a	0.55a
<i>S. bullata</i>	0.60a	0.40a

Df=1, 8;  $F=1.72$ ;  $P=0.202$

A



B



Figure 5-1. Containers for parasitoids conditioned for 48 h on different host pupal types, with Petri plates containing three choices of pupal host as well as a blank control Petri plate. A) Containers were arranged in a randomized block pattern to control for variations in light intensity, temperature and surroundings. B) During the experiment, plastic tops were securely fastened onto containers to prevent escape of insects.



Figure 5-2. Containers for parasitoids that emerged from different pupal types, with Petri plates containing two choices of pupal host as well as a blank control Petri plate. Containers were arranged in a randomized block pattern to control for variations in light intensity, temperature and surroundings

## CHAPTER 6 Y-TUBE OLFACTOMETER EXPERIMENTS

### Introduction

For most insects, olfaction serves as a vital form of communication. The necessity of locating and identifying odors is important for insects that cannot utilize visual or auditory cues to locate mates, food, hosts and water (Hallem et al. 2006). Female insects, especially those whose mates must fly great distances for mating opportunities, may give off pheromone compounds to signal their location to males, e.g., the tobacco hornworm moth *Manduca sexta* L. (Daly et al. 2001) and silkworm moths (Lepidoptera: Saturniidae) (Hansson 1995). Olfactory cues are provided by different insect species, vertebrates and plants as well as an insect's own species. The use of kairomone compounds is well-documented in entomology (Johnson and Triplehorn 2004), mainly to attract insects to a source. The gall wasp, *Antistrophus rufus* Gillette, for example, has demonstrated a sex-mediated development schedule in which adult males emerge from galls prior to female emergence to increase their chances of mating with a virgin female (Tooker et al. 2002). Males of *A. rufus* locate unemerged females by following olfactory cues produced by stems of their host plants (genus *Silphium*) rather than from olfactory cues provided by the females of *A. rufus*. Honey bees (Hymenoptera: Apidae) provide another classic example of olfaction-based resource location (Reinhard et al. 2004), in which a scent (e.g., from food) is associated with a location. Bees exposed to a scent specific to a location will return again to that location.

Research on the relationship between associative learning and olfaction in insects has yielded interesting examples of how strongly an initial exposure to a scent that is tied to a reward (e.g., food or host source) can affect the future behavior of that insect. Foraging members of the yellowjacket species *Vespula germanica* (Fab.) will, when simultaneously exposed in their nest

to a food source and a scent, will preferentially visit a source outside the nest with that particular scent profile, compared to a control (Jandt and Jeanne 2004). While much entomological data suggest that associative learning is an important component of behavior for many insects, those species with a life history strategy dependent on a host (e.g., parasitoids) present the question of whether prior exposure to a host will affect the later behavior of that insect. Specifically, it is unknown for many parasitoids whether close contact with a host during the larval stages will affect the adult behavior of that insect with regards to locating and ovipositing in that same host.

Many species of parasitoid wasps have demonstrated measurable changes in behavior following exposure to chemicals associated with hosts or host-fostering habitats. In many cases, the surroundings of the host may provide the olfactory stimuli, rather than the host itself. Such odors may be produced by plants that a host feeds on, decaying plant or animal matter that host larvae develop in, or host frass. Both of the parasitoid wasps, *Dibrachys cavus* (Walker) and *Roptrocerus xylophagorum* (Ratzeburg) (Hymenoptera: Pteromalidae), for example, demonstrated a greater preference for the frass of their respective host insects than for the hosts (Chuche et al. 2006; Sullivan et al. 2000, respectively).

The host insect itself may be the source of volatile chemicals that attract parasitoids, rather than the surroundings of the host. The Diapriid parasitoid *Trichopria drosophilae* Perkins was found to be attracted to kairomones emitted by the anterior spiracles of its host, *Drosophila melanogaster* L. (Romani et al. 2002). Host recognition by *Muscidifurax raptor* Girault and Sanders involves chemicals emitted by its host *Musca domestica* L. rather than from the feeding and rearing medium of the host (in this case, manure) (McKay and Broce 2003). In an olfactometer, *M. raptor* preferred the odor of host pupae alone over the odor of manure. When

both manure and pupae were presented in one arm of the olfactometer, *M. raptor* was not attracted to the odors from that arm (McKay and Broce 2003).

Utilizing an olfactometer to document insect choice-making behavior is a common technique in associative learning research with parasitoids. de Jong and Kaiser (1991), for example, demonstrated that fruit fly larval parasitoid *Leptopilina boulardi* Barbotin et al. (Hymenoptera: Eucoilidae) females exposed to an artificial odor in conjunction with an oviposition experience would demonstrate a much stronger choice in an olfactometer for the scent they were exposed to during stimulus/reward conditioning compared to naïve female parasitoids, as well as to controls which were not classically conditioned. In this study, artificial odors were considered ones not naturally produced by the host or plants fed on by the host. Later studies with *L. boulardi* indicated that when females were exposed to multiple odors at different times, they demonstrated a strong preference for all odors compared to naïve parasitoids, with the strongest preference for the most recently-learned host (de Jong and Kaiser 1992). Learning by parasitoids to preferentially follow an odor has been documented in *Cotesia marginiventris* Cresson (Turlings et al. 1989), *Leptopilina heterotoma* (Thomson) (Papaj and Vet 1990) and *Microplitis croceipes* (Cresson) (Lewis and Tumlinson 1988). In the case of *M. croceipes*, which is a parasitoid of *Heliothis zea* (Boddie), female wasps could be successfully conditioned to locate odors which are not naturally associated with their host, and in fact are unattractive, such as vanilla (Lewis and Tumlinson 1988).

Because *Trichopria nigra* (Nees) has demonstrated a host range consisting of multiple fly species (chapter 3) as well as significant changes in behavior following conditioning to a host pupal type (chapter 5), the first objective was to determine whether conditioning females of *T. nigra* to a host species' pupae creates a stronger preference to odors from that host they were

exposed to, compared to naïve female parasitoids (e.g., females not previously exposed to pupal odors). The second objective was to determine whether the length of time females of *T. nigra* are exposed to a potential host influences their host-seeking behavior. Lastly, it was unknown whether rearing *T. nigra* on different host species affects female host preference, and whether the speed and strength of naïve female *T. nigra* response to host odors change with age.

## **Materials and Methods**

### **Study Site**

All experiments were conducted in a glass Y-tube olfactometer (Agricultural Research Systems, Gainesville FL) (Figure 6-1) approximately 24 cm in length, with a tube diameter of approximately 5 cm. Parasitoids were prevented from escaping during experiments by closing the main arm of the Y-tube with a glass entry capsule containing a mesh opening (for air escape). Flow rate was 0.3 L per minute (LPM) for all experiments. A clean air source was provided by the air system at the CMAVE facility, which was supplied via a nalgene tube and filtered by bubbling through H<sub>2</sub>O before flowing through the arms of the olfactometer. To negate the possible effect of phototaxis, lighting was diffuse, being provided by four 32 W fluorescent lights positioned centrally approximately 1.2 m above the olfactometer. Room temperature was 22-24°C.

### **Parasitoids**

For all experiments except 6-3 (rearing effect on host choice), female *T. nigra* adults from both the Russian and Kazakh strains were pooled immediately following emergence. Only female parasitoids were utilized in experiments since Chapter 5 data suggest that male parasitoids do not react significantly in a closed arena to volatiles from host pupae. Strains were pooled by placing rearing containers (see chapter 3) in a refrigerator at ~13°C for five minutes, then aspirating females from the bottom of both containers with a small handheld aspirator. All

aspirated females were gently blown into a Petri plate bottom to pool both strains, and gently dropped into gelatin capsules for holding prior to experiments. For each repetition, five females were counted and placed into an empty gelatin capsule (size “0”) and were introduced to the olfactometer by gently tapping the gelatin capsule against a hard surface to stun the parasitoids and prevent them from flying away before placement in the olfactometer, and twisting it open with the parasitoid-containing half shaken into the terminal end of the olfactometer. The entry capsule was then placed into the terminal end of the olfactometer, preventing escape.

Controls were run prior to each experiment, consisting of five replicates of five naïve female parasitoids introduced to the olfactometer with an air flow of 0.3 LPM. Both arms were empty, and parasitoids were monitored for bias toward either arm of the olfactometer. The Y-tube apparatus and associated stimulus capsules (Figure 6-2) were not washed between replicates, as one arm of the Y-tube was designated for one of two stimuli, and attraction to that arm did not change between replicates. Between experiments, all glass parts of the Y-tube apparatus were washed with detergent and rinsed with tap and deionized water, respectively. All pieces were allowed to air-dry, to avoid accidental marking of glass pieces with volatiles from drying materials and fabrics.

For all experiments, strength of response was determined as being the proportion of females which made a choice by traveling at least halfway down one arm of the olfactometer.

### **Experiment 6-1: Effect of Three Days of Conditioning on Host Choice**

*T. nigra* adults (both strains) were aspirated from rearing containers within 12 h of adult emergence and divided into three groups. A surplus (<1000) of parasitoids was collected to ensure an adequate number of surviving females. Approximately 150 cc of 2-d-old *Sarcophaga bullata* Parker pupae and 150 cc of *Stomoxys calcitrans* (L.) pupae were each placed into ca. 500 ml paper cups. One group of parasitoids introduced to each container for conditioning. The third

group was placed into an empty paper cup as a control. Containers were covered with muslin and secured with rubber bands. A streak of honey and a water-dampened cotton ball placed atop the muslin cover provided food and water. The paper containers were placed in a 25°C incubator ( $\pm$  0.4°C, 70% RH) and *T. nigra* females were allowed 3 d to associate host pupal odors with oviposition (i.e., conditioning).

After the 3-d conditioning interval, parasitoids were knocked down by refrigerating the paper cups for several minutes ( $\sim$ 13°C) and then aspirating females from the inside of the cups. Four replicates of five females each (i.e., five females at a time), conditioned to *S. bullata* pupae, were introduced for 3 min into the olfactometer with ca. 1.0 g of either *S. bullata* or *S. calcitrans* pupae (ca. 2 d old) in the arms, respectively. Pupae had, immediately prior to experimentation, been collected from colonies at the USDA's CMAVE facility, washed, dried gently, and placed into the attractant chambers at the ends of the arms. Four replicates of five females each (e.g., five at a time), conditioned to *S. calcitrans* pupae, were introduced for 3 min into the olfactometer with ca. 1.0 g of *S. bullata* and *S. calcitrans* pupae (ca. 2 d old) in the arms, respectively. Additionally, four replicates of five unconditioned females each were introduced to the olfactometer with ca. 1.0 g each of *S. bullata* and *S. calcitrans* pupae (ca. 2 d old) in the arms. First choice was noted for each female within a replicate, as well as time required for each female to make a first choice.

Females which did not choose either arm of the olfactometer within 3 min were considered non-responders and not included in data analysis.

### **Experiment 6-2: Host Choice Response after 1 d, 3 d, and 5 d of Conditioning on *S. bullata* Pupae**

*T. nigra* adults (both strains) were aspirated from rearing containers within 12 h of adult emergence and divided into two groups. A surplus (<1000) of parasitoids was collected to ensure

an adequate number of surviving females. Approximately 150 cc of 2-d-old *S. calcitrans* pupae were placed into a ca. 500 ml paper cup. One group of parasitoids was placed in the container with *S. calcitrans* pupae, and the other was placed in an empty paper cup as a control. Containers were covered with muslin and secured with rubber bands. A streak of honey and a water-dampened cotton ball placed atop the muslin cover provided food and water. The paper containers were placed in a 25°C incubator ( $\pm 0.4^\circ\text{C}$ , 70% RH). After 1 d, females were aspirated from each container in groups of five (eight groups total) with a manual aspirator and placed in gelatin capsules for experimentation, and the remaining parasitoids returned to the incubator.

Eight replicates of five females each, conditioned to *S. calcitrans* pupae, were introduced for 3 min into the olfactometer with ca. 1.0 g of *S. calcitrans* pupae in one arm and a control arm not containing a stimulus. First choice was noted for each female within a replicate, as well as time required for each female to make a first choice. Females which did not choose either arm of the olfactometer within 3 min were considered non-responders and not included in data analysis. Eight more sets of five females were collected at 3 d and 5 d after parasitoids were exposed to pupae, and the above experimental procedure repeated.

### **Experiment 6-3: Host Choice by Parasitoids Reared on Different Hosts**

*T. nigra* adults were reared on pupae of *S. bullata* and *S. calcitrans*, respectively (for detailed rearing methodology, see Chapter 3). Within 12 h of parasitoid emergence, the ~500-ml paper cups of pupae and newly-emerged parasitoids were provided with food and water via a streak of honey and a water-dampened cotton ball placed atop the secured muslin covers. The paper cups were placed in a 25°C incubator ( $\pm 0.4^\circ\text{C}$ , 70% RH) for 48 h to allow for cuticle hardening and sexual maturity of parasitoids.

Six replicates of five females each, reared on *S. bullata* pupae, were introduced for 3 min into the olfactometer with ca. 1.0 g each of *S. bullata* and *S. calcitrans* pupae (ca. 2 d old) in the arms, respectively. Six replicates of five females each, reared on *S. calcitrans* pupae, were introduced for 3 min into the olfactometer with ca. 1.0 g each of *S. bullata* and *S. calcitrans* pupae (ca. 2 d old) in the arms. Additionally, four replicates of five unconditioned females each were introduced to the olfactometer with ca. 1.0 g each of *S. bullata* and *S. calcitrans* pupae (ca. 2 d old) in the arms. First choice was noted for each female within a replicate, as well as time required for each female to make a first choice.

#### **Experiment 6-4: Response of Unconditioned Parasitoids to Hosts During Five Days Post-Emergence**

*T. nigra* adults were aspirated from rearing containers within 12 h of adult emergence and placed into a ca. 500 ml paper cup which was then covered with muslin, secured with rubber bands, and food and water provided via a streak of honey and a water-dampened cotton ball placed atop the muslin cover. A surplus (<1000) of parasitoids was collected to ensure an adequate number of surviving females. The paper cup was placed in a 25°C incubator ( $\pm 0.4^\circ\text{C}$ , 70% RH) for 1 d to allow for cuticle hardening and sexual maturity of parasitoids. After 1 d, six replicates of five females were aspirated from the paper container and introduced for 3 min into the olfactometer with ca. 1.0 g of *S. calcitrans* pupae (ca. 2 d old) in one arm and a control (empty) arm. First choice was noted for each female within a replicate, as well as time required for each female to make a first choice. The above procedure was repeated on days two, three, four and five after emergence.

#### **Statistical Analysis**

For Experiment 6-1, G tests were conducted to determine if the sampling distribution of frequency of choices female parasitoids made in the olfactometer was random, or if there were

significant differences between the distributions of the three treatments. Parasitoids conditioned to *S. calcitrans* were compared to parasitoids conditioned to *S. bullata*. Additionally, parasitoids conditioned to *S. calcitrans* as well as parasitoids conditioned to *S. bullata* were individually compared with the controls. G test statistics were rounded to two significant figures, and an alpha level of  $P < 0.05$  was considered significant.

For Experiment 6-2, two sets of G tests were conducted to test for variance in the frequency of choices made by unconditioned and *S. calcitrans* conditioned parasitoids with respect to time and treatment. *S. calcitrans* conditioned parasitoids were compared to unconditioned controls at one day, three days and five days. Individually, both *S. calcitrans* conditioned parasitoids and unconditioned controls were subjected to two G tests: one comparing day one choices for that treatment with day three choices, and another comparing day one choices with day five choices. G test statistics were rounded to two significant figures, and an alpha level of  $P < 0.05$  was considered significant.

For Experiment 6-3, a single G test was conducted to test for variance in the frequency of choices made by *S. calcitrans* conditioned parasitoids versus *S. bullata* conditioned parasitoids. Additionally, for parasitoids that did respond to a stimulus in the olfactometer, a two-way ANOVA was conducted on the mean response times of each treatment to the two choices, to determine if any significant differences in response time emerged as a result of rearing host (SAS Institute 2000).

For Experiment 6-4, G tests were conducted to test for differences in response strength (e.g., number of parasitoids that chose pupae or the control arm) over time. Four comparisons were made, examining cumulative choices made by female parasitoids on days one and two, days one and three, days one and four, and days one and five. G test statistics were rounded to

two significant figures, and an alpha level of  $P < 0.05$  was considered significant. A two-way ANOVA was conducted to test for any significant differences in mean response time and mean response strength for the five days the experiment was conducted. Response strength was determined as the proportion of five females that made a decision within 3 min for each repetition.

## Results

Unconditioned female parasitoids preferred *S. calcitrans* pupae over *S. bullata* pupae (Table 6-1). Host conditioning made a significant difference in the number of *T. nigra* females which responded to their conditioning host versus an unfamiliar host (Table 6-5). Comparing *S. calcitrans* conditioned parasitoids to unconditioned controls ( $G=0.02$ ;  $df=1$ ) did not demonstrate any significant difference in strength of response, nor did *S. bullata* conditioned parasitoids compared to the unconditioned ones ( $G=2.16$ ;  $df=1$ ).

Comparing *S. calcitrans* conditioned parasitoids with their unconditioned counterparts did not yield any significant differences on day one, day three or day five (Table 6-2). Comparing the cumulative numbers of *S. calcitrans* conditioned parasitoids responding to pupal odors on days one and three yielded a significantly greater response on day three ( $G=4.70$ ;  $df=1$ ), although comparing day one and day five responses of *S. calcitrans* conditioned parasitoids did not ( $G=3.42$ ;  $df=1$ ). Additionally, no significant changes were observed between responses of unconditioned parasitoids when days one and three and one and five were compared ( $G=0.75$ ;  $0.27$  respectively;  $df=1$ ). The greatest increase in response rate was seen in *S. calcitrans* conditioned parasitoids between day one and day three (Table 6-2).

For parasitoids reared on two different hosts, there was no significant correlation between host rearing type and pupal choice in the olfactometer (Table 6-5). While a greater number of *S.*

*calcitrans* conditioned parasitoids chose their natal pupal type over an unfamiliar stimulus (Table 6-3), *S. bullata*-conditioned parasitoids did not choose their natal pupal type.

The number of naïve parasitoids responding to odors from *S. calcitrans* pupae increased from day one to day three, then decreased slightly to day five (Table 6-4). While response strength did not change with respect to time in Experiment 6-4, the mean response time did change significantly ( $P=0.04$ ;  $df=4$ ) over the five days. Comparing the strength of unconditioned *T. nigra* female response during the first five days after adult emergence yielded no significant differences in G tests (Table 6-5). The fastest mean response time for female parasitoids to *S. calcitrans* pupae was on day three.

### Discussion

The results of Experiment 6-1 agree with the first arena experiment conducted in Chapter 4; i.e., that short-term conditioning of *T. nigra* females to volatiles produced by host pupae does indeed influence their host-seeking behavior to preferentially search for hosts of that exposure type. Interestingly enough, while 48 h of female *T. nigra* exposure to one of two pupal types created a preference for conditioning host, experiment 6-3 does not corroborate with the second arena experiment conducted in Chapter 4. While in Chapter 4 we found that rearing does influence host selection in an enclosed arena, the female parasitoids who were reared on *S. calcitrans* and *S. bullata* pupae did not demonstrate a significant preference for their rearing host when presented with those pupal types as choices in a Y-tube olfactometer. de Jong and Kaiser (1992) suggest that the most recent exposure to positive stimulus in association with an odor has the greatest influence on insect behavior, compared to earlier conditioning experiences. From an ecological perspective, this assumption makes sense because, in the wild, a female parasitoid who encounters multiple positive stimuli (e.g., an odor in association with an oviposition event) is most likely to receive a reward if she seeks out the most recent location or type of stimulus.

Several explanations exist for the lack of differences in the behavior of unconditioned parasitoids compared to those conditioned in *S. calcitrans* pupae. One possibility is that utilizing clean, dried pupae does not provide a strong enough attractant for parasitoids, who are instead seeking out odors of the environment associated with that host, such as odors associated with larvae, their frass, or their larval rearing substrate (Sullivan et al. 2000). Additional research is needed to determine whether *T. nigra* utilizes a hierarchy of stimuli which includes host habitat and proximity of host larvae in addition to cues that direct them to host pupae.

For many parasitoids, a combination of cues is necessary to successfully locate hosts. This may be a combination of olfactory cues, of which some are attractive at a distance and others at close range (Morehead and Feener 2000), or a combination of visual and olfactory cues. *Diachasmimorpha juglandis*, for example, a parasitoid of *Rhagoletis* flies, locates its host via the *Rhagoletis* food source, walnut fruit husks (Henneman et al. 2004). In an olfactometer, *D. juglandis* can discern between intact walnuts and those which have been damaged by *Rhagoletis*. However, when presented with visual rather than olfactory cues, such as when both mechanically damaged and host-damaged walnut husks were presented, *D. juglandis* had little success discerning between husks with hosts and husks without (Henneman et al. 2004). Clearly, visual clues are often important for host location at a distance, luring parasitoids close enough to locate olfactory cues.

For pupal parasitoids, it is possible that a combination of odors contributes to host-seeking behavior. However, for parasitoids with a broad host range, such as *T. nigra*, the question arises of whether parasitoids naturally seek out multiple, and possibly quite different, odors. McKay and Broce (2003) found that, for *Muscidifurax zaraptor* Kogan and Legner, the odors emitted from house fly puparia were much more attractive than the manure house fly

larvae naturally occur in. Furthermore, female parasitoids could not differentiate manure with a combination of pupae and manure as a stimulus, when presented with both choices in a y-tube olfactometer. *T. nigra*, with a host range which encompasses flies that develop in a variety of substrates and media (e.g., manure and carrion), the smaller and more closely-related milieu of odors emitted from related host species may be the primary attractant, rather than a broad spectrum of complicated odors.

The increase in response time to *S. calcitrans* pupae by unconditioned parasitoids in a Y-tube olfactometer through day three, followed by a decrease in both response rate and response time, indicates that the optimal oviposition time for this species is likely around three days post-emergence. If *T. nigra* does not mate inside the host (as is suggested due to spatial constraints), the delay in optimal host location behavior may be explained by *T. nigra* requiring this amount of time to mate and complete hardening of the cuticle. Clearly, further research is required to examine whether conditioning to host pupae does indeed override preference for natal host type, as well as whether post-emergent conditioning to the same pupal type as the natal host type strengthens the response of female *T. nigra* searching for a host to oviposit in. In addition, it remains to be determined whether olfactory cues associated with a host, such as the media in which *S. calcitrans* and *S. bullata* develop, are stronger attractants for female *T. nigra* compared to the odors associated with pupae alone.

Table 6-1. First choices made by female parasitoids conditioned for 3 d on either *S. calcitrans* or *S. bullata* pupae in a Y-tube olfactometer. The two choices presented in the olfactometer arms were *S. calcitrans* and *S. bullata* pupae, respectively.

Conditioning host	Cumulative number of parasitoids attracted to	
	<i>S. calcitrans</i>	<i>S. bullata</i>
No conditioning	30	17
<i>S. calcitrans</i>	45	16
<i>S. bullata</i>	26	28

Table 6-2. First choices made by female parasitoids conditioned on *S. calcitrans* pupae and tested at 1 d, 3 d and 5 d against unconditioned female parasitoids of the same age. The two choices presented in the olfactometer arms were *S. calcitrans* pupae and a blank arm, respectively.

Conditioning host	Day after emergence	Cumulative number of parasitoids attracted to	
		<i>S. calcitrans</i>	blank
No conditioning	1	19	15
No conditioning	3	28	7
No conditioning	5	24	7
<i>S. calcitrans</i>	1	14	6
<i>S. calcitrans</i>	3	25	6
<i>S. calcitrans</i>	5	26	8

Table 6-3. First-turn choices made by 3-d old female parasitoids reared on either *S. calcitrans* or *S. bullata* pupae and run in a Y-tube olfactometer. The two choices presented in the olfactometer arms were *S. calcitrans* and *S. bullata* pupae, respectively.

Rearing host	Cumulative number of parasitoids attracted to	
	<i>S. calcitrans</i>	<i>S. bullata</i>
<i>S. calcitrans</i>	13	6
<i>S. bullata</i>	12	11

Table 6-4. First-turn choices made by female parasitoids run in a Y-tube olfactometer at 24-h intervals after emergence. Day 1 was defined as 24 h post-emergence. ANOVA F values are listed below means. Numbers marked with an asterisk are considered significant ( $P < 0.05$ ).

Day	Cumulative number of parasitoids attracted to		Response time to <i>S. calcitrans</i> (s)
	<i>S. calcitrans</i>	blank	
1	19	9	44.0 (4.9)
2	23	6	52.6 (5.6)
3	25	6	35.7 (3.9)
4	32	7	36.5 (3.7)
5	23	10	49.2 (6.1)
ANOVA <i>F</i>			2.54*
df=4,1; $P < 0.05$			

Table 6-5. Results of G tests for the four olfactometer experiments, comparing likelihood ratios between variables. Numbers marked with one asterisk are considered significant at  $P < 0.05$ , while numbers marked with two asterisks are considered significant at  $P < 0.01$ .

Experiment	Variables	Comparison: conditioning, time	G
6-1	Host conditioning on <i>S. calcitrans</i> or <i>S. bullata</i>	<i>S. calcitrans</i> v. <i>S. bullata</i>	8.04**
		<i>S. calcitrans</i> v. no conditioning	1.23ns
		<i>S. bullata</i> v. no conditioning	2.52ns
6-2	Host conditioning on <i>S. calcitrans</i>	Day 1: <i>S. calcitrans</i> v. no conditioning	1.07ns
		Day 3: <i>S. calcitrans</i> v. no conditioning	<0.01ns
		Day 5: <i>S. calcitrans</i> v. no conditioning	<0.01ns
	Host conditioning on <i>S. calcitrans</i> , with time	Unconditioned, day 1 v. day 3	4.69*
		Unconditioned, day 1 v. day 5	3.42ns
		<i>S. calcitrans</i> , day 1 v. day 3	0.75ns
6-3	Rearing on <i>S. calcitrans</i> or <i>S. bullata</i> pupae	<i>S. calcitrans</i> , day 1 v. day 5	0.27ns
		<i>S. calcitrans</i> v. <i>S. bullata</i>	1.15ns
6-4	Unconditioned, per day post-emergence	Unconditioned, day 1 v. day 2	0.97ns
		Unconditioned, day 1 v. day 3	1.27ns
		Unconditioned, day 1 v. day 4	1.79ns
		Unconditioned, day 1 v. day 5	0.02ns

At  $P < 0.05$ ,  $G \geq 3.8$ , and at  $P < 0.01$ ,  $G \geq 6.6$ .

A)



B)



Figure 6-1. Y-tube olfactometer used in experimentation. A) Air flow and humidity were controlled for by a regulator bubbling air through distilled water. B) Up to two choices were presented to parasitoids in screened chambers that allowed air flow of volatiles. Choice was counted as the first arm a parasitoid moved halfway down, regardless of future turns or time spent in that arm.

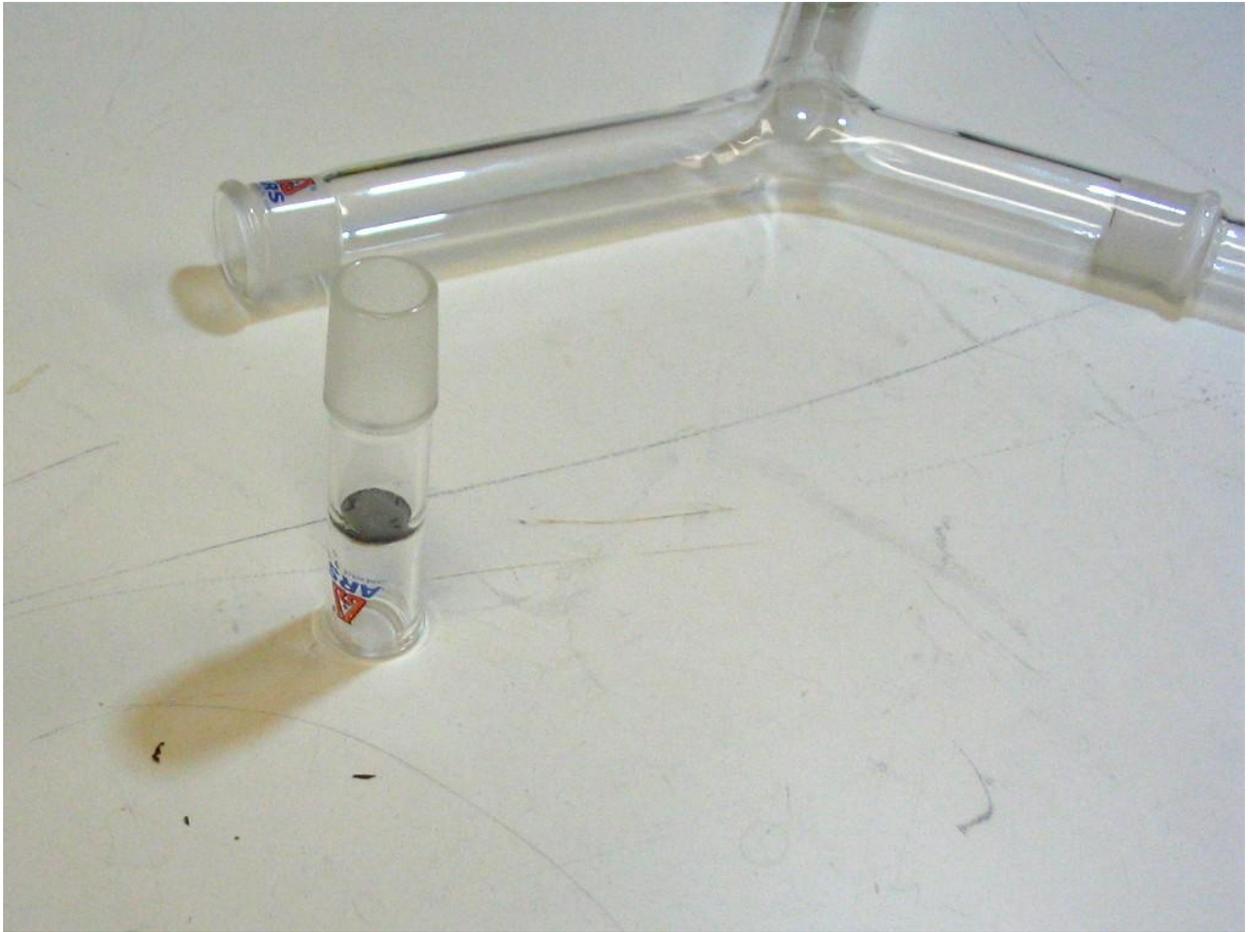


Figure 6-2. Y-tube olfactometer arms and entry capsule. Between experiments, all glass pieces were washed, rinsed twice and allowed to air-dry.

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## BIOGRAPHICAL SKETCH

Kimberly Marie Ferrero was born on March 18, 1983, the first child of Patti A. Ferrero and Dr. Frank A. Ferrero. Her father delivered her at Miami Mercy Hospital, where he was a heart surgeon. Kimberly is the oldest of five children, and has two younger sisters, Ashley and Francesca, as well as two younger brothers, Christopher and Shawn. A first generation Italian-American, she grew up in Miami, Florida and attended high school at Lake Mary High in Orlando, Florida. As a young child, her love of science blossomed under the guidance of many wonderful teachers, so that at the age of seventeen she became one of only two students to teach honors biology at her high school.

Following high school, she began attendance at the University of Florida, and in 2001 completed her Bachelor's degrees in Anthropology and Zoology, with a focus on the evolutionary biology of primates. Her years at the University of Florida allowed her to take part in research travel to places such as Indiana University in the United States, St. Andrews University in Scotland, and Suriname in South America. A summer internship in the final year of her undergraduate education, at the University of Florida's Entomology and Nematology department, convinced Kimberly to remain at the university to pursue a Master's degree in medical and veterinary entomology, with an emphasis on control of medically important Diptera (flies and mosquitoes).

Kimberly subsequently spent the next two years working at the United States Department of Agriculture's on-campus Center for Medical, Agricultural and Veterinary Entomology, and her love of public health issues was rewarded when she was permitted to conduct her Master's research on a little-known parasitoid from Eastern Europe that attacks and kills filth flies that are common worldwide. She has given numerous talks and lectures on the importance of control of arthropod disease vectors, and has taught Introductory Entomology at the University of Florida

as a Graduate Teaching Assistant. She spends much of her free time collecting insects in the wilderness of north-central Florida, and enjoys swimming, kayaking, sailing, rock climbing and gourmet cooking on a graduate student budget.

As a final note, Kimberly's inspiration for pursuing a career in the sciences is attributed to her father, Dr. Ferrero. A gifted doctor and scientist with degrees in biology and physics, his unwavering support of her education and insistence that women can be influential figures in the sciences has allowed her to complete this thesis. Although Dr. Ferrero passed away one year before seeing his daughter obtain the first of her graduate degrees, his passion for helping people through a greater understanding of the natural world lives on in his daughter.