2021

CHARACTERIZING THE HOST USAGE AND DEVELOPMENT OF ORMIA LINEIFRONS (DIPTERA: TACHINIDAE)

Kyler J. Rogers

CHARACTERIZING THE HOST USAGE AND DEVELOPMENT OF *ORMIA LINEIFRONS* (DIPTERA: TACHINIDAE)

A Thesis

Presented to the Faculty of the Department of Biological Sciences

Murray State University,

Murray, Kentucky 42071

In Partial Fulfillment

of the Requirements for the Degree

of Master of Biology

By

Kyler J. Rogers

July 2021

ACKNOWLEDGEMENTS

There are many people I would like to thank for helping me throughout my academic endeavors. First, I need to thank my parents, Kathy, and Robert Rogers, for their boundless encouragement and supporting me to further my education. I am forever grateful to them. Next, I thank Shilo and Felipe Lopez, as well as Noah and Josiah, for their love and kindness, it meant more to me than they realize. Thank you. I would also like to thank my committee members Drs. Oliver Beckers, Laura Sullivan-Beckers, Michael Flinn, Christopher Mecklin, and Howard Whiteman. Their guidance and support were paramount for me in my quest to develop as a scientist.

I am deeply indebted to the biology faculty at Murray State University, they were extremely helpful and ensured to answer any questions I had. To Dr. James E. O'Hara, thank you so much for all your help and insight in *Ormia* identification and ecology, you were such a great resource for me, I owe you. I would also like to acknowledge the funding that helped me tremendously throughout my master's program. These include Howard Whiteman and the Watershed Studies Institute at Murray State University for supporting my project and the SUPERB program.

ABSTRACT

Insect communication systems are strongly driven by the evolution of signals or signal preferences. These systems rely on a signaler to truthfully emit signals for receivers to interpret. Often, these signals are conspicuously broadcasted. Conspicuous signals involved in animal communication are intended to attract mates, however, these signals are often exploited by eavesdroppers. Thus, many communication systems experience natural selection and sexual selection acting in opposite directions. New adaptations can arise in response to selective pressures, such as eavesdroppers, leading to co-evolving systems between eavesdroppers and hosts, for example. Understanding these systems can provide valuable insight into how unintended receivers can shape the evolution of communication systems. The katydid genus, *Neoconocephalus,* relies on acoustic communication for mating, whereby males will produce acoustic calls to attract mates. This communication system is exploited by the eavesdropping tachinid fly, *Ormia lineifrons,* and suffers high levels of parasitism. These parasitoids are a strong selective force on their hosts because they inevitably kill the host within seven to nine days after infestation. The natural history of *O. lineifrons* and *Neoconocephalus sp.* interactions lack characterization and is the primary focus of my thesis.

In Kentucky, *O. lineifrons* is multivoltine and co-occurs throughout multiple *Neoconocephalus* seasons. Interestingly, four *Neoconocephalus* species were parasitized by *O. lineifrons,* three of which are newly discovered hosts. *Ormia lineifrons* larvae had higher development success rates in *N. velox* than in *N. triops,* respectively. Additionally, *N. robustus* and *N. triops* pupae were both, on average, significantly heavier than *N. velox*

pupae. As *Ormia lineifrons* clutch size increased in *Neoconocephalus* hosts, pupa mass significantly decreased. I found no differences in the mean clutch size or development time of *O. lineifrons* among the host species. The parasitoid, *Ormia lineifrons,* imposes selective pressure on multiple *Neoconocephalus* species in Kentucky. This pressure has the potential to limit the reproductive success for *N. triops, N. velox, N. robustus* and *N. nebrascensis* during their breeding seasons. This is the first detailed study outlining the host usage, activity, and development of *Ormia lineifrons.*

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Natural and sexual selection are integral components in the theory of evolution (Darwin 1859). Individuals within a population possess a variety of traits that can be selected for or against by natural selection, sexual selection, or both. Notably, natural selection can be driven by predation and/or parasitism (Darwin 1859). Intuitively, traits that are more conspicuous to predators would experience strong selective pressure, especially if the traits decrease an individual's chance for survival.

The interactions between parasites and their hosts are diverse and complex (see reviews in Godfray 1994; Brodeur & Boivin 2004). Understanding the interactions between hosts and parasites has provided much needed insight, especially in the context of how natural selective pressures can shape the evolution of host populations (Vinson & Iwantsch 1980; Civantos et al. 2005; Brodeur & Boivin 2004). Some key insights gained from these interactions are that parasites can exhibit host synchronization, parasites can be gregarious or solitary, and parasites use different strategies for development (Alphen & Vet 1986; Allen et al. 1999). Synchronization coinciding with host activity is paramount for successful parasitism. Selection would act against parasites that are active during times when there are no available hosts. For example, parasites can diapause to compensate for times where hosts are inactive (Godfray 1994; Calero-Torralbo & Valera 2008). Solitary parasites use only a single offspring per host, whereas gregarious parasites allocate multiple offspring among hosts. Parasitizing a host more than once is

referred to as superparasitism and can occur in both solitary and gregarious parasites, however this behavior is more common in gregarious parasites (review in Brodeur & Boivin 2004). Parasite development can be approached by using the idiobiont-koinobiont dichotomy (Askew & Shaw 1986). Idiobiont parasites cease the development of the host after infection, whereas koinobiont parasitoids allow the host to continue feed and grow after infection (Brodeur & Boivin 2004).

Communication systems, particularly in insects, involve signalers and receivers wherein the signaler (i.e., males) broadcasts a signal for mate attraction and the receiver (i.e., females) uses the information contained in the signal to find and assess potential mates (Bradbury & Vehrencamp 2011). Signals can be broadcasted in various forms, such as, chemical, visual, vibrational, and/or acoustic (Burk 1988; Wyatt 2014). Each of these four modalities have shown extensive exploitation by eavesdroppers and parasites in their respective system (reviews by Burk 1982 & Zuk & Kolluru 1998; also see Harris & Todd 1980; Allan et al. 1996; Huigens et al. 2009). Parasites can eavesdrop and exploit mating signals as cues to find their hosts (Zuk & Kolluru 1998; Lehmann 2003). This is primarily because they have co-evolved closely with the communication system and are specialized at finding a host using their sexual signals (e.g., Lehmann & Heller 1998).

Within the order Diptera, Tachinidae is a diverse family wherein each of the flies are known to be endoparasitoids (develop inside the host) of arthropods (Stireman III et al. 2006; Feener Jr & Brown 1997). Parasitoids are distinct from parasites in that they rely on the host for development and inevitably kill their host after emergence, whereas parasites only need their host to complete a specific step of their life cycle (Lehmann 2003; Stireman III et al. 2006). For example, male crickets of the genus, *Gryllus*,

acoustically broadcast conspicuous calls to attract silent females. Females use these signals to assess and find stationary males to mate with, likewise, the eavesdropping parasitoid fly, *Ormia ochracea*, uses this cue to locate male hosts (Cade 1975; Walker & Wineriter 1991). Female *O. ochracea* deposit mobile planidia larvae on or near a host, and then the larvae penetrate the host through the interscleral membranes (Léonide 1969; Brodeur & Boivin 2004; Stireman III et al 2006). These endoparasitoids take about nine days to develop, whereafter the larvae emerge from the host and kill it, followed by the larvae pupation and then metamorphosing into adult flies (Walker 1993). Parasitoids have adapted to their hosts to increase their fitness. For example, the hearing systems of the eavesdropping parasitoid fly *O. ochracea* adapted to respond to its hosts calls by being most sensitive in the frequency range of the cricket's call (Robert et al. 1992). Alternatively, there may be superparasitism by some insect parasitoids because it is thought to be an adaptive strategy (Alphen & Visser 1990). Superparasitism can be adaptive in nature if a parasitoid has higher patch search time, lacking good-quality hosts or if host availability has been reduced (Godfray 1994; Brodeur & Boivin 2004). However, superparasitism, as the name suggests, is a method where many offspring are allocated to a single host and these offspring experience increased conspecific competition for host resources. This leads to a commonly observed tradeoff in parasitoids: offspring tend to be smaller in size as clutch size increases (Lehmann 2003).

The male traits exhibited by individuals in a population can be shaped by both female preference (sexual selection; Gray & Cade 1999; Wagner & Reiser 2000; Simmons et al. 2001; Lehmann et al. 2001; Beckers & Wagner 2012) and eavesdropping parasites (natural selection; Walker & Wineriter 1991; Adamo et al. 1995; Gray et al.

2007). To compensate for the selective pressure induced by *O. ochracea*, novel host adaptations have arisen in cricket populations. In Hawaii, *O. ochracea* uses the cricket *Teleogryllus* as a host. Zuk and colleagues (2006) observed a new 'silent' wing morph adaptation that appeared in response to the strong pressure exerted from parasitism on one island and it subsequently spread to other islands. As a result of the inability to call, crickets evolved a new strategy for mate finding, in which both male and female crickets search for a mate (Zuk et al. 2006). Moreover, Beckers & Wagner (2018) found that *G. lineaticeps* males produce more attractive calls (for conspecific females) but also more dangerous calls (attractive to parasitoids) to increase mating before parasitism occurred. Interestingly, female *G. lineaticeps* have been shown to prefer more dangerous male songs, even though these songs increased their chances to be parasitized (Beckers & Wagner 2018). The males producing these more dangerous signals increase the female's fecundity through their seminal fluids (Beckers & Wagner 2018). Although host adaptations in response to parasitism are well documented (also see Allen 2000; Zuk et al. 2006), information on the adaptations of the parasite remain sparse.

Koinoboint endoparasitoids, such as *O. ochracea*, rely heavily on their host species for development (i.e., Crosskey 1965; Allen et al. 1999; Adamo et al 1995; Beckers et al. 2011; Brodeur & Boivin 2004). Although much is understood about the complex dynamics and interactions in the *O. ochracea* system, a closely related system lacks comprehensive study. The eavesdropping parasitoid, *O. lineifrons*, is known to parasitize *Neoconocephalus triops* in Florida (Burk 1982) with the potential to parasitize other *Neoconocephalus* species. It has been shown that *N. triops* suffers perennially high

levels of parasitism by *Ormia lineifrons* (Burk 1982; Lehmann 2003). Nothing else is known about this evolutionary exciting species interaction.

My thesis addresses two main questions related to the interaction between *Neoconocephalus sp.* and *O. lineifrons*. Who are the hosts and how does the development of *O. lineifrons* differ between them? Chapter I bolsters the current understanding of the interaction between *O. lineifrons* and its katydid hosts. The only information available on *O. lineifrons* comes from the Florida population that uses *N. triops* as a host in both the spring and the fall and parasitism reaches up to 100% (Burk 1982). In Kentucky, *N. triops* is univoltine (one generation; spring) and there is no information regarding how often and when *Ormia lineifrons* is active during the year. Assuming a similar life history of *O. lineifrons* in Kentucky, I hypothesize that *O. lineifrons* is likely bivoltine or multi-generational and follows a similar cycle of parasitizing the *N. triops* populations here in Kentucky, as seen in Florida. Since *N. triops* is univoltine in Kentucky, I predict that *O. lineifrons* utilizes other hosts species besides *N. triops*. To test my hypothesis, I surveyed *Neoconocephalus* populations in Kentucky for two years to identify who the hosts were, how high the parasitism rate of each host was, and how many generations *Ormia lineifrons* has here.

Chapter II focuses on the developmental success of *O. lineifrons* larvae within each of the *Neoconocephalus* hosts. This is important because of various potential host adaptations, such as host size (Godfray 1994), that differ between species and affect fly development. Therefore, I hypothesize that development of *O. lineifrons* larvae differs across host species. Finally, not only host species but also clutch size can affect developmental success of the parasitoid. Typically, a larger clutch size reduces the

developmental success of the larvae inside the host (Allen 1995; Welch 2006). Thus, I hypothesize that a larger clutch size in a host will reduce *O. lineifrons* success rate. To answer these questions of parasitoid development, I measured the clutch size, developmental time, pupal mass, and the developmental success for *O. lineifrons* pupae.

CHAPTER I

PARASITISM OF *NEOCONOCEPHALUS* KATYDIDS BY THE PARASITOID FLY *ORMIA LINEIFRONS*

Abstract

Conspicuous advertisement signals of insects are intended to attract potential mates; however, these signals can be exploited by eavesdropping predators. These signals can lead to natural selection and sexual selection acting on a signaler in opposite directions, facilitating evolution in the exploited communication system and lead to new counter adaptations. The species interactions (i.e., predator/parasite & host) in these systems are driven by selective pressure, promoting diversification of individuals in the populations. Thus, these arms races between signaler and eavesdropper have a high potential to introduce diversification in communication systems that directly affect fitness and provide excellent study systems for evolutionary ecology.

In this study, I quantify the parasitoid-host interaction of the parasitoid fly, *Ormia lineifrons,* and its *Neoconocephalus* katydid hosts. I surveyed the host-use of *O. lineifrons* over a two-year period in Kentucky and determined host species usage, the parasitism rates for each katydid host, and the number of fly generations for each year. Based on data from Florida, I predicted that *O. lineifrons* in Kentucky is bivoltine and uses at least one other species besides the known host *N. triops.*

Four of the six surveyed *Neoconocephalus* species were parasitized and killed by *O. lineifrons*. Of these, *Neoconocephalus velox, N. robustus,* and *N. nebrascensis* are newly identified hosts. In Kentucky, I found that *Ormia lineifrons* has three generations

per year (multivoltine) and each generation used different host species. Additionally, my data suggest that *Ormia lineifrons* likely enters diapause at the end of the year. The parasitism rate of *Neoconocephalus* hosts peaked between 40% and 100% across species. The parasitoid exerted selective pressure through lethal parasitism on multiple katydid species, especially *N. triops* and *N. velox*. The synchronization of *Ormia lineifrons'* generations with *Neoconocephalus* activity across the different breeding seasons likely represents co-evolution that has occurred in this system. Further, these systems may be actively co-evolving, largely to mitigate the selective pressure from parasitism.

Introduction

Communicating insects typically produce signals to attract mates for reproduction (Gerhardt & Huber 2002). Male signals tend to be conspicuous to optimize conspecific female attraction and because females frequently prefer males with more conspicuous signals (e.g., Ryan & Keddy-Hector 1992; Wagner 1996; Kotiaho et al.1996; Bernal et al. 2006). The males producing these signals have been shown to provide higher direct and/or indirect benefits to the female (reviews in Andersson 1994; Wagner 2011). However, these conspicuous signals can also attract unintended, eavesdropping predators and parasites to the calling male (reviews in Zuk & Kolluru 1998; McGregor 2005). As a result of these eavesdroppers exploiting the communication systems, natural and sexual selection act on signals and signaling behavior in opposing directions, leading to a range of adaptations to reduce the detrimental effects of parasitism on fitness (e.g., Zuk et al. 2006; Beckers & Wagner 2018; Cade et al. 1996; de Silva et al. 2014).

The evolutionary consequences of parasitism have been well-documented in the interaction between the field crickets *Gryllus spec.* and *Teleogryllus oceanicus* and the parasitoid fly, *Ormia ochracea* (Cade 1975). Tachinid flies (family: Tachinidae) lack a rigid ovipositor (Stireman III et al. 2006) and typically place mobile planidia larva on and/or around the host (e.g., Adamo et al. 1995; Cade 1975). Cricket males produce acoustic mating signals to attract female conspecifics and female *O. ochracea* use these signals to locate male crickets that are used as hosts for their parasitic planidia larvae (Cade 1975; Adamo et al. 1995; Walker & Wineriter 1991). *Ormia ochracea* exerts selective pressure on field crickets because the larvae kill their host within ten days (Adamo et al. 1995). Since the larvae kill the host, *O. ochracea* is considered a parasitoid. *Ormia ochracea* uses different cricket hosts in various geographic ranges and a growing body of literature has described various adaptations hosts have exhibited in response to extensive parasitism (Belanger & Zuk 2015). For example, these adaptations range from a substantial reduction of singing (Zuk et al. 2006) in *Teleogryllus oceanicus* to the evolution of fly-preferred mating songs in *Gryllus lineaticeps* (Beckers & Wagner 2018), highlighting how parasitism can lead to diversification across multiple host species. The *Ormia ochracea* - field cricket system has produced multiple important insights foundational in parasite ecology and evolution (e.g., Zuk et al. 1993; Zuk et al. 1998; Zuk et al. 2006; Tinghitella & Zuk 2009; Beckers & Wagner 2018), only a few other acoustic eavesdropping parasitoid and host systems have been previously identified and explored (review in Zuk & Kolluru 1998).

In contrast to *Ormia ochracea,* research on other eavesdropping parasitoids in the genus, such as *O. brevicornis*, *O. depleta,* and *O. lineifrons,* has been very limited (but

see Nutting 1953; Mangold 1978; Burk 1982; Shapiro 1995). My study focuses on the interaction between *O. lineifrons* (Sabrosky 1953) and its *Neoconocephalus* katydid hosts. Like the parasitized crickets used as hosts by *O. ochracea*, male katydids produce acoustic signals for mate attraction and *O. lineifrons* parasitizes these katydids (Burk 1982). The life cycle of *O. lineifrons* is like that of *O. ochracea* (Burk 1982). The availability of hosts for larval development is crucial for *Ormia lineifrons* reproductive success. My study aims to further explore the ecological interaction between *O. lineifrons* and its *Neoconocephalus* hosts.

In western Kentucky, multiple *Neoconocephalus* species are reproductively active between spring and fall (SINA 2020). Among those katydids, *Neoconocephalus triops* (Linnaeus 1758) is a known host *of Ormia lineifrons* in Florida (Burk 1982). The Kentucky population of *N. triops* displays only one generation per year (i.e., in the spring; OMB pers. obs.), while the *N. triops* population in Florida display two generations per year (Whitesell 1974). Other univoltine *Neoconocephalus* species present in Kentucky are *N. velox* (Rehn & Hebard 1914), *N. nebrascencis* (Bruner 1891), *N. bivocatus* (Walker, Whitesell, Alexander 1973), *N. robustus* (Scudder 1862), *N. retusus* (Scudder 1878), *N. palustris* (Blatchley 1893), and *N. exiliscanorus* (Davis 1887), however their parasitism status is not known. *Ormia lineifrons* also parasitizes meadow katydids of the genus *Orchelimum* (Shapiro 1995), which are not addressed in this study.

The objectives of this study are to determine (i) which *Neoconocephalus* species are used by *O. lineifrons* as hosts, (ii) what the parasitism rate for each host species is, and (iii) to describe the life cycle of *O. lineifrons* in Kentucky. This study is the first comprehensive report on host use throughout the *O. lineifrons* breeding season.

In Florida, *Ormia lineifrons* parasitizes *Neoconocephalus triops* (Burk 1982) in the spring and the fall, I hypothesized that *O. lineifrons* parasitizes *N. triops* in Kentucky during the spring. Since *N. triops* is univoltine in Kentucky, *O. lineifrons* is either univoltine as well or is bivoltine and parasitizes one or more species after the *N. triops* season in the spring. The abundance of multiple katydid species occurring in the spring and fall, along with the presence of *O. lineifrons* (OMB. & KJR pers. obs.), provides a great opportunity to answer basic questions about the natural history of this parasitoid and host(s) system.

Materials and Methods

Collection of Neoconocephalus katydids

I collected katydids from an area within an approximately 30-mile radius around Murray, KY (36.6103º N, 88.3148º W) between April and September of 2019 and March and October of 2020. I collected katydids between two to four times each week throughout the duration of each species' breeding season. I searched for calling males on nights that were warmer than 12ºC at dusk and sampled for at least two hours. To find the katydids, I drove on country roads with open windows, listening for the loud and conspicuous calls of *Neoconocephalus* males. I used these songs to locate the calling males for collection. I collected six *Neoconocephalus* species during the 2019 and 2020 seasons and checked each for parasitism. I collected a total of 386 katydids of the following species in sequence of their seasonal occurrence: *N. triops* (2019: N=85; 2020: N=69), *N. velox* (2019: N=18; 2020: N=15), *N. nebrascensis* (2019: N=12; 2020: N=27), *N. robustus* (2019: N=47; 2020: N=37), *N. bivocatus* (2019: N=12; 2020: N=8), and *N.*

retusus (2019: N=25; 2020: N=31). I attempted to collect *N. palustris* (Blatchley 1893) and *N. exiliscanorus* (Davis 1887), both are present in Kentucky (SINA 2020), however I did not find either species in the sampled area. I continued to sample areas where I collected each species for at least another week after calling ceased to ensure that the breeding season of the species was concluded and not temporarily interrupted. I collected the katydids by hand and placed them into a centrifuge tube (50mL; Falcon brand) for transport to the lab. Each katydid was transferred within a day of capture to an individual cage with food and water to determine the parasitism status.

Each of the *Neoconocephalus* species produces a species-specific mating call. I used these calls for species identification by recording and analyzing the calls in the lab and comparing them to published data by Büttner (2002) and SINA (2020). To differentiate *N. robustus* from *N. bivocatus*, I measured the width of the stridulatory fields on the forewings of the males for each species (Walker et al. 1973; Walker et al. 1993). I recorded males of all sampled species for identification and to establish a record of the call characteristics of the hosts of *O. lineifrons* in Kentucky (Table 2-1).

Animal care

I kept each insect in a separate cage (15.57 x 23.19 x 15.25 cm, height x length x width) in an incubator (PR505755L; Thermo Fisher Scientific) with a light/dark cycle of 15.5/8.5h and coinciding 'day' and 'night' temperatures of 26/22ºC, respectively. The day length and temperatures correspond approximately to a long summer day in Murray, Kentucky. Each cage had a plastic lid with mesh screen glued to the underside, preventing roaming *O. lineifrons* larvae from escaping the cage. The humidity in the incubator ranged between 65-85%. I provided each katydid with organic apple, rolled

oats and high-calcium cricket food (Fluker) for food, and water gel (Tasty Worms Nutrition, Inc.) as a source of water. I sprayed each cage daily with water and replaced food and water gel every two days.

Ethics statement

All animal care and experimental procedures adhered to the ASAB/ABS guidelines for the use of animals in research, the legal requirements of the U.S.A., and all guidelines of Murray State University.

Call recordings

I acclimatized field-collected katydids to a reversed day-night cycle in an incubator for at least two days before recording the animals during the dark portion of the cycle. The incubator conditions were the same as described above. I used the set-up and procedures outlined in Beckers et al. (2019) for call recordings and analysis. In brief, I placed each katydid in a separate custom-built mesh recording cage inside a Styrofoam cooler (53.3 x 40.6 x 26.7 cm, length x height x width; Loboy) that was lined on each side with 5-cm sound absorbing acoustic foam (HushFoam, HFW-2; Silent source) and 1.3 cm-thick mineral fiber ceiling tile (Armstrong). I separated coolers by approximately 50 cm distance and the coolers were placed in a custom-built semi-anechoic chamber (3.43 x 3.15 x 2.10 m, length x height x width). Each recording cooler was outfitted with a thermometer (15-077-27; Fisherbrand) and a tie-clip microphone (ATR3350; Audio-Technica). Each microphone fed into a separate recording channel through an interface (US 1800; Tascam) and recorded onto a hard drive of a PC (Optiplex 780, Dell) using the software Cubase (version 5.0 SL; Steinberg). Call recordings were saved as WAV files with a sample rate of 48 kHz and a bit rate of 16. I haphazardly broadcasted WAV file of

the calls of each species to be recorded (files downloaded from SINA 2020) from an elevated position above the coolers using a MP3 player (Clipjam, Sandisk), amplifier (RX4109, Sherwood), and a loudspeaker (PCB4BK, Pyle) to stimulate calling. The broadcasted stimuli were calibrated at 80±2 dB SPL at 30 cm (peak amplitude, fast, C-weighing). I turned the broadcasts off as soon as a male started singing. I recorded all males at 25.4 ± 0.5ºC.

All *Neoconocephalus* species produce songs by rubbing their wings against each other producing a continuous train of sound pulses. The sound pulses are interrupted by intervals of silence (pulse intervals). Some species (i.e., *N. triops, N. bivocatus, N. retusus*) space subsequent pulses by alternating short and long intervals, resulting in a pairing of pulses into pulse pairs, or double pulses. *Neoconocephalus robustus, N. velox*, and *N. nebrascencis* produce a train of evenly spaced pulses with a single pulse pattern. In addition, species differ in their specific pulse rate (i.e., the number of pulses per second) and in their carrier frequency (Büttner 2002; Beckers & Schul 2008). Additionally, *N. triops* (Whitesell & Walker 1978) and *N. nebrascensis* (Büttner 2002) show a gross temporal call structure by interrupting long trains of sound pulses with intervals of silence, i.e., they produce 'versed' calls instead of 'continuous' calls. I analyzed the pulse pattern, pulse rate, call structure and dominant frequency of all sampled species.

I used the custom software Song_X (BSE software) to down-sample (12kHz) and analyze the call recordings of each collected katydid. I measured the above mentioned fine temporal call features of approximately 1s of call recording (>170 pulses). To determine the verse duration and verse interval of *N. nebrascensis* and *N. triops*, I

analyzed at least 10 adjacent verses and verse intervals of each recording, using the software Audacity (version 2.3.3. for Macintosh; Audacity Team). I used the software Audacity also to determine the dominant frequency of an approximately 1s long recording of each animal (Hanning window, sample size of 512).

Parasitism status

Ormia lineifrons is attracted to the calling song of male *Neoconocephalus* katydids, and since *Neoconocephalus* females do not produce calls, I collected and determined the parasitism status only for male katydids. I checked the parasitism status of each collected animal in the laboratory daily by inspecting the cage and the katydid for the presence of roaming larvae or fly pupae and if the katydid was dead. Twenty-four hours after I found a dead katydid in its cage, I dissected its thorax and abdomen under a dissecting scope (S6-RLT; Richter Optica) to check for any larvae that failed to emerge from the host. This procedure allowed me to distinguish whether the katydid died because of parasitism or from an unrelated reason (e.g., age). On average, *O. lineifrons* larvae emerge from its hosts between seven to nine days (Burk 1982). I kept all animals for at least six weeks after collection in the laboratory and checked their parasitism status daily before I euthanized the katydids by freezing (-20ºC for 48h). I calculated the parasitism rate for each week by dividing the number of katydids parasitized of each species by the total number of katydids collected of that species during that week. The first week corresponds to the first day I heard the species calling in the field. As the season for each species progressed, the number of active, and therefore collected, animals naturally declined due to parasitism, age, predation, and other natural reasons.

I kept the pupae in centrifuge vials (50mL; Falcon brand) on moist cotton at the base of the tube at the same incubator temperatures and photoperiods as the katydids (see above) until they metamorphosed into adult flies. The lids of the tubes had holes to allow for gas exchange. I checked the cotton daily for bacterial growth and dryness. I sprayed the cotton with water when needed and exchanged it when I detected fungal growth. I sent a sample of 15 *Ormia* flies that emerged from collected *Neoconocephalus* hosts from 2019 and 2020, to Dr. James O'Hara at the Agriculture and Agri-Food Canada for species identification. This sample covered all host species that are reported here, and I provided flies from multiple individuals of each *Neoconocephalus* species. Dr. O'Hara confirmed that all flies were *O. lineifrons*, which also means that Kentucky is the northernmost reported record of *O. lineifrons'* range to date (O'Hara et al. 2020).

Statistical Analysis

I compared the duration of successful development from pupa to adult among the fly generations using a two-factor ANOVA using the software JMP (14.2.0). I used 'year of collection' (2019, 2020), 'generation' (first, second, third generation) and the interaction 'year of collection x generation' as fixed effects and 'developmental time' as response variables. For katydids that were host to more than one successfully developing pupa, I averaged the developmental time across the successful pupae for that host, resulting in one data point for each host.

Results

I collected data of parasitism rates of six species of *Neoconocephalus* in western KY between 2019 and 2020. The breeding season across all species ranged from late

March to early October (**Table 1-1, Figure 1-1**). In both years, the first reproductively active species was *N. triops* followed by *N. velox*. Later in the season, *N. robustus*, *N. bivocatus* and *N. nebrascensis* were reproducing and partially overlapped with each other. The last species that I collected was *N. retusus* (**Figure 1-1**). I detected parasitism by *O. lineifrons* in *N. triops* (total number of parasitized/all collected individuals for 2019: 23/85 and 2020: 26/69), *N. velox* (2019: 11/18; 2020: 13/15), *N. robustus* (2019: 8/47; 2020: 4/37), and *N. nebrascensis* (2019: 3/12; 2020: 4/27). However, I did not detect parasitism of *N. bivocatus* (2019: 0/12; 2020: 0/8) or *N. retusus* (2019: 0/25; 2020: 0/31) in either year of collection. Parasitism rates across both years peaked for *N. triops* and *N. velox* at 100% and for *N. nebrascensis* and *N. robustus* at 57% and 50%, respectively (**Figure 1-1**).

My laboratory rearing of *O. lineifrons* larvae that emerged from field-collected katydids indicated three distinct fly generations per year (**Figure 1-2**). The first lab generation (G1; **Figure 1-2**) were flies that developed in *N. triops* and the second lab generation (G2; **Figure 1-2**) were flies that developed in *N. velox*. The adult flies of the third generation were flies using *N. robustus* and *N. nebrascensis* as hosts (G3; **Figure 1-2**). This third generation was less pronounced than the first two peaks in both years. The pupa developed directly, i.e., without a diapausing stage, into adult flies. Development of pupa to adult fly ranged on average (± SD) between 11.71 ± 0.95 days and 12.71 ± 1.38 days across generations and years. I did not detect any significant difference in developmental times among the three generations (ANOVA: $F_{2,52} = 0.57$, $p = 0.57$), the two years (ANOVA: $F_{1,52} = 1.79$, $p = 0.19$), or the interaction between the year of collection and the generations (ANOVA: $F_{2,52} = 1.74$, $p = 0.19$).

Discussion

I found that *O. lineifrons* had three generations per year and exerted selective pressure on four of the six sampled *Neoconocephalus* species in Kentucky. Parasitism rates reached high levels, ranging from 50 to 100%, depending on host species and year. Neither *N. bivocatus* nor *N. retusus* were parasitized by *O. lineifrons*.

Synchronization of parasitoid generations with host occurrence

The rearing of *O. lineifrons* showed that the larvae developed directly into flies without diapausing, resulting in three generations per year in Kentucky. Due to the direct development, each generation of flies coincided with a different seasonal host or hosts. The first generation consisted of offspring of *O. lineifrons* that parasitized *N. triops* in the early spring. *Neoconocephalus triops* is a tropical species that extended its range into temperate North America (Whitesell 1974; Beckers & Schul 2010) and, in contrast to the other *Neoconocephalus* species in Kentucky, diapauses as adult and begins reproducing as soon as temperatures rise in the early spring (Whitesell 1974). The flies parasitizing *N. triops* represent overwintering individuals from the third or last generation of the previous year. Parasitism of *N. triops* reached 100% within two to three weeks in both years. Some field-collected unparasitized *N. triops* lived up to forty-six days in the lab following the cessation of *N. triops* field calling field (unpublished data), therefore it is unlikely the population crashed due to age. The consistent increase of parasitism to 100% in both years suggests that fly parasitism plays a major, if not the most important, role in the crash of the *N. triops* population. While collecting, I observed one to two silent satellite males were within 30 cm of a calling male on five occasions. The parasitism rate of the collected satellite males was low (14.3%, n = 7), suggesting that the satellite

behavior might have been a behavioral tactic to reduce the parasitism risk (Cade 1975; Cade 1984; Bertram et al. 2004), rather than the result of parasitism-related injury affecting calling activity (Zuk et al. 1995).

The only published data on parasitism rates of hosts of *O. lineifrons* are those from the population in Florida. In Florida, *N. triops* has a bivoltine life cycle with a spring and a fall generation and both generations are parasitized by *O. lineifrons* (Burk 1982). However, in Florida the fall generation experienced much higher parasitism (90 - 100%) than the spring generation (38 - 54%; Burk 1982). I observed the reversed pattern in Kentucky where *N. triops* is univoltine and has only the spring generation (KJR pers. obs.). In contrast to the Florida population, the spring and not the fall generation in Kentucky experienced high parasitism rates (100%, **Figure 1-1**). The host species active during the fall in Kentucky (i.e., *N. robustus* & *N. nebrascensis*) did not reach the parasitism rates observed in Florida in the fall (max. 57% vs. 100%). This comparison suggests that the parasitoid life cycle and thus the parasitoid/host dynamics differ between populations and provides opportunities for comparative ecological studies.

In contrast to *N. triops*, parasitism rates were high in both years in the first weeks of *N. velox* calling activity. Female *O. ochracea* live as adults on average about twenty-three days as adults in the lab (Wineriter & Walker 1990) and probably less in the field. Assuming a similar life expectancy for *O. lineifrons*, it is unlikely that the female flies that parasitized *N. triops* were still alive when *N. velox* became active (**Figure 1-1**). My rearing indicated that adult flies started hatching from pupae in weeks 6 (2019, **Figure 1-2**) and 7 (2020, **Figure 1-2**) and would have started to become gravid about two to three weeks later (Paur & Gray 2011). Thus, gravid females were likely searching for hosts

when *N. velox* started to become active in week 11 (2019, **Figure 1-2**) and week 13 (2020, **Figure 1-2**), explaining the high parasitism rates of *N. velox,* even at the beginning of its season.

Ormia lineifrons' flies of the third laboratory generation started to turn adult in week 14 (2019, **Figure 1-2**) and week 15 (2020, **Figure 1-2**) and *N. robustus* and *N. nebrascensis* became acoustically active in week 16 or 17 in each year (**Figure 1-1**). This timing suggests that the flies emerging from *N. velox* hosts likely parasitized *N. robustus* and *N. nebrascensis* in the fall. Note that in 2020, *N. velox* activity ended very closely to the beginning of *N. robustus* and *N. nebrascensis* activity, raising the possibility that some late flies of the *N. velox* parasitism peak could have been alive and used these two katydid species as hosts.

In 2019, I detected an increase in parasitism at the very end of the *N. robustus* season at week 20 (**Figure 1-2**). At this point, adults of the third lab generation that used *N. robustus* and *N. nebrascensis* as hosts could have become gravid and searching for hosts. The first adults of the third generation emerged from the pupae in week 19 (**Figure 1-2**), possibly explaining this resurgence of parasitism in *N. robustus*.

In both years, adult *O. lineifrons* flies of the third generation would have been gravid when *N. retusus* was active. I did not detect parasitism of this species in either year. It is unlikely that this third fly generation was an artifact of rearing protocol since these conditions were comparable to field conditions in July and August when the pupae of the third peak developed. The average maximum and minimum temperatures for this time in 2019 and 2020 were 31.5 - 20.3ºC and 32.7 - 19.2ºC, respectively (www.wunderground.com), whereas my rearing temperatures were 26.0 - 22.0ºC. It is

possible that *O. lineifrons* used different hosts in the fall, such as the katydid *Orchelimum nigripes* (Shapiro 1995) or other species present in Kentucky (SINA 2020). It may also be that these adults forgo reproduction and instead diapause as suggested for *O. ochracea* (sensu Paur & Gray 2011), possibly explaining the early spring activity of *O. lineifrons* when it attacks the adult diapausing *N. triops*.

Importantly, the synchronization of fly activity with host occurrence is essential for the reproductive success of *O. lineifrons.* The temporal decoupling of host/parasitoid occurrence can have a detrimental ripple effect on the fitness of subsequent fly generations. Thus, I propose that there is likely strong selection on the synchronization between the parasitoid and the occurrence of the host species.

Natural selection and possible adaptations of host species

My data indicate that *O. lineifrons* in Kentucky exerts selective pressure to a varying degree on multiple host species. This suggests that there are at least four separate host-parasitoid arms races taking place in Kentucky, possibly leading to different adaptations, and thus introducing phenotypic variation in each host species.

Ormia lineifrons interacts on two levels with the host. First, the adult fly recognizes and localizes the host to successfully deposit the larvae onto it. Second, the larvae need to feed and interact primarily with the immune system inside the host to successfully develop (e.g., Adamo et al. 1995). Host adaptations could take place on either or both levels, i.e., avoiding being detected by the fly and/or attacking the larvae once infected. As a result, host species might evolve a more efficient immune response to the parasitoid larvae. Selection might also favor desynchronization of the breeding season of *Neoconocephalus* hosts with that of the parasitoid to reduce parasitism, as potentially

seen in the early occurrence of *N. triops*. Other possible adaptations of *Neoconocephalus* host species could be reduced calling activity (Vélez & Brockmann 2006), satellite behavior (Cade 1975; Cade 1984), increased grooming to remove planidia larva (Vincent & Bertram 2010), increased caution (Lewkiewicz & Zuk 2004), shift of calling activity (Cade et al. 1996), and/or calling from protected positions (KJR pers. obs.).

In contrast to the semi-independent evolution of the host species to the parasitoid, *O. lineifrons* needs to evolve a broad set of adaptations to utilize the range of host species, which is exemplified by the requirement to recognize a range of host calls (**Table 1-1**). It is possible that host recognition is broadly tuned, accepting the displayed variation in one (e.g., pulse rate) or multiple call characteristics of the host species. Alternatively, it is also plausible that developmental plasticity through imprinting on the calls of the most prevalent host (Paur & Gray 2011) in each fly's generation could provide flexible host specificity.

Lack of parasitism of N. retusus and N. bivocatus

I did not detect parasitism of *N. retusus* and *N. bivocatus* by *O. lineifrons* in either year, even though flies were active in the area where these katydids called. For both species, the calls overlapped in their temporal characteristics with those of parasitized species (**Table 1-1**), suggesting that the temporal pattern may not explain the lack of fly attraction. In contrast to *N. bivocatus*, the carrier frequency of *N. retusus* was substantially higher (14.4 kHz) than that of all parasitized species (≤ 10.6 kHz) and might be outside the tuning of *O. linefrons'* auditory system. In addition, carrier frequencies of *Neoconocephalus* calls above 10 kHz are much more attenuated over distance in their natural grass land habitat than lower frequencies (Schul & Patterson 2003), which should

further decrease detectability of *N. retusus* calls by the fly but also the female katydids. Further experiments are necessary to test whether this tradeoff in detectability is the result of parasitism.

The co-occurrence of *N. bivocatus* with two host species, i.e., *N. robustus* and *N. nebrascensis*, may have substantially reduced the parasitism risk for *N. bivocatus*. Especially with the sympatric and loud *N. robustus* males that call from elevated positions (Walker et al. 1973; KJR & OMB pers. obs.), might divert *O. lineifrons* attraction from *N. bivocatus*. However, my small sample suggests a small population size of *N. bivocatus* in western Kentucky and further research is needed to better understand the effect of *O. lineifrons* parasitism on this species.

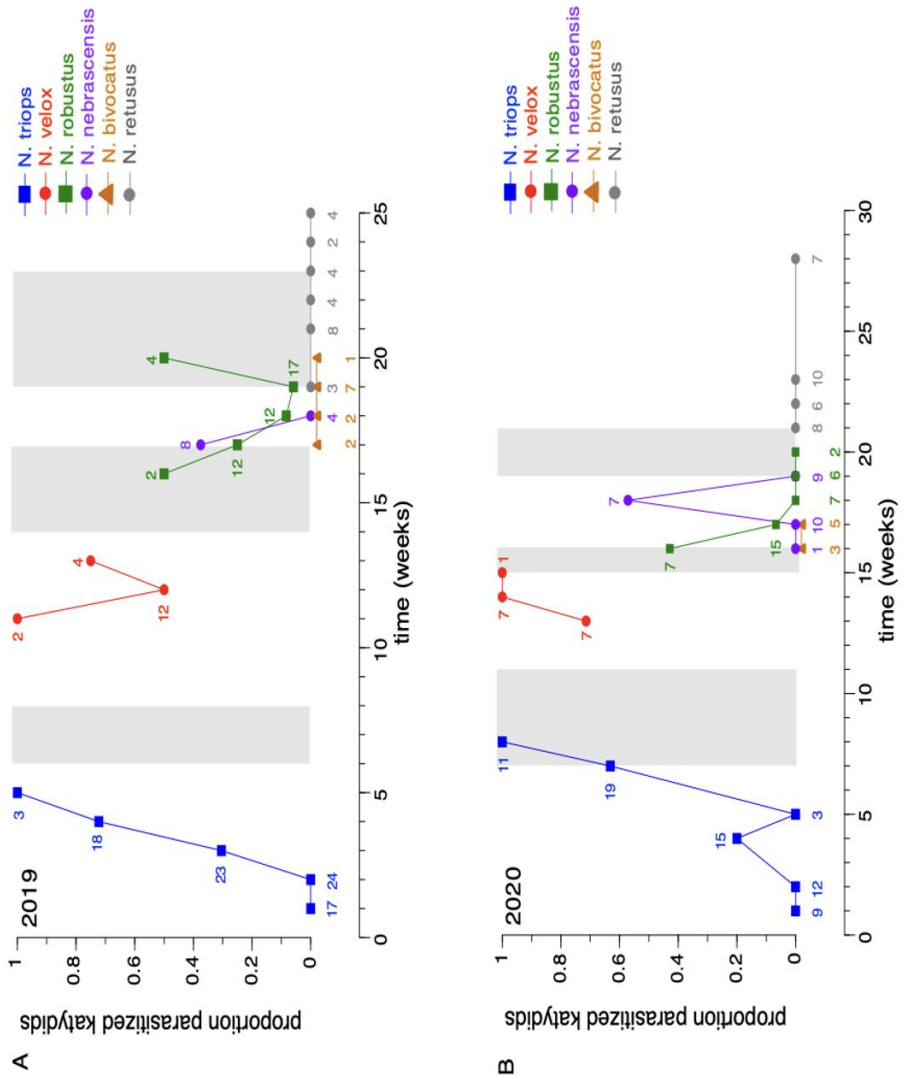

Figure 1-1: Parasitism rates for the six *Neoconocephalus* species (A) for 2019 and (B) for 2020 over each reproductive season. In panel A, the start of the season (week 1) is April 3, 2019, and in panel B, March 28, 2020. Numbers next to symbols indicate the sample size (N). The hatched fields indicate the emergence of adult *O. lineifrons* based on lab rearing.

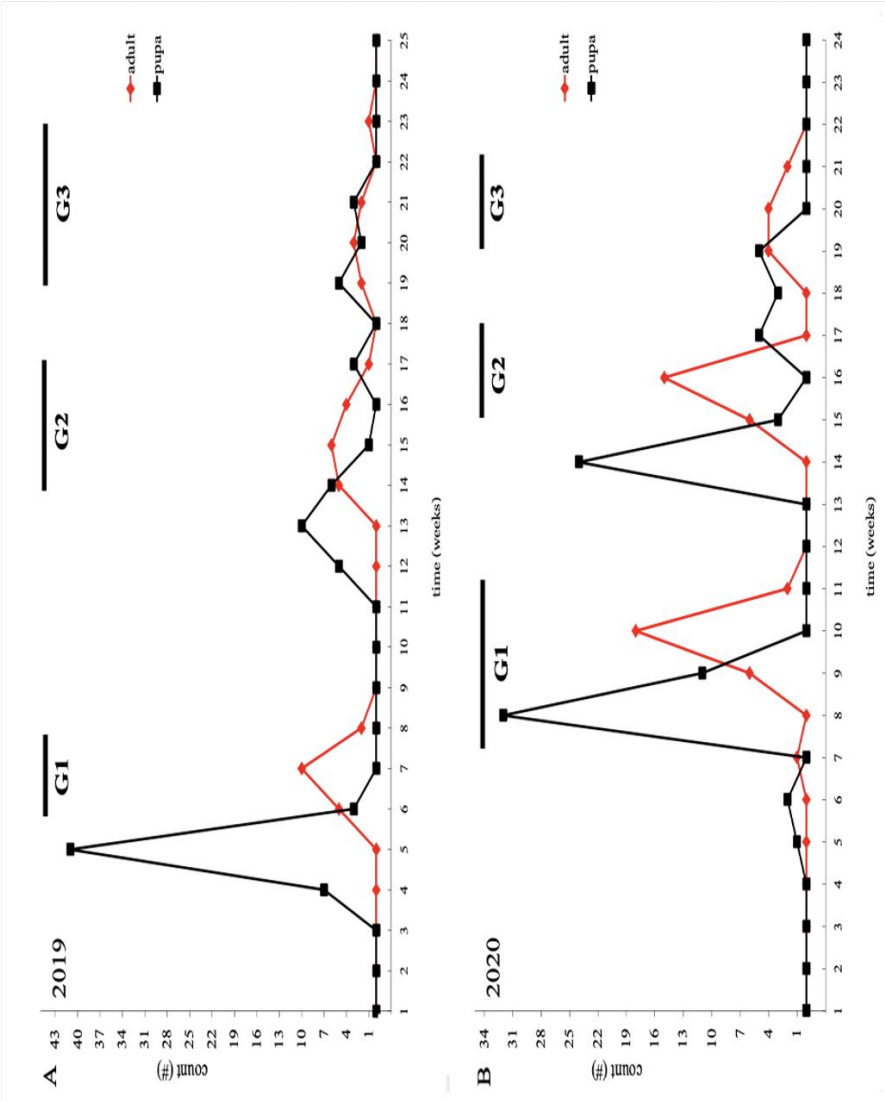

Figure 1-2: Counts of *O. lineifrons* pupa and adult flies that developed from the pupae that emerged from *Neoconocephalus* hosts collected in the field in (A) 2019 and in (B) 2020. The start for the 2019 season (week 1) was April 3 and March 28 for the 2020 season, i.e., the weeks for each year shown in the figure are aligned with the weeks shown in Figure 1. Black bars on top of each figure indicate fly generations (G) and are based on the dates of emergence of at least one adult flv in the lab.

Table 1-1: Call character, breeding season, and max. parasitism rates of six *Neoconocephalus* species. Pulse pattern is the production of single pulses (SP) or double-pulses (DP), i.e., evenly vs. unevenly spaced pulses of the mating calls. Calls that are rhythmically interrupted by (verse) intervals of silence are versed calls, whereas calls without interruption are continuous (Cont.) calls. Sample sizes (N) of recorded and analyzed males are indicated below each call character.

Species	**Season** 2019 (top) 2020 (bott.)	**Pulse pattern**	**Pulse rate (pulses/sec ± SD)**	**Peak frequency (kHz ± SD)**	**Verse duration (sec ± SD)**	**Verse interval (sec ± SD)**	**Max. Parasitism rate (%)** 2019; 2020
N. triops	4/3 – 5/7 3/28 – 5/20	DP	180.5 ± 11.7 (n=31)	10.6 ± 0.8 (n=31)	1.29 ± 0.2 (n=31)	0.05 ± 0.03 (n=31)	100%; 100%
N. velox	6/15 – 7/2 6/24 – 7/11	SP	176.4 ± 10.0 (n=5)	9.3 ± 1.2 (n=5)	Cont.	Cont.	100%; 100%
N. robustus	7/22 – 8/14 7/11 – 8/19	SP	209.8 ± 10.4 (n=32)	6.9 ± 0.8 (n=32)	Cont.	Cont.	50%; 43%
N. nebrascensis	7/30 – 8/11 7/11 – 8/4	SP	186.7 ± 8.3 (n=10)	9.3 ± 0.7 (n=10)	1.55 ± 0.3 (n=10)	1.02 ± 0.2 (n=10)	38%; 57%
N. bivocatus	7/29 – 8/14 7/11 – 7/20	DP	170.9 ± 4.4 (n=8)	10.0 ± 0.6 (n=8)	Cont.	Cont.	0%; 0%
N. retusus	8/11 – 9/20 8/19 – 10/8	DP	176.6 ± 8.8 (n=24)	14.4 ± 1.0 (n=24)	Cont.	Cont.	0%; 0%

CHAPTER II

ASSESSING DEVELOPMENTAL DIFFERENCES IN HOST USAGE BY *ORMIA LINEIFRONS*

Abstract

In insect communication, many mating signals are broadcasted conspicuously and are vulnerable to exploitation. New counter adaptations can arise in response to the selective pressure exerted by exploiting eavesdroppers. The eavesdropping tachinid, *Ormia lineifrons* parasitizes multiple *Neoconocephalus* hosts in Kentucky. I present data on the parasitoid interactions in each of the host systems.

I hypothesized that development of *O. lineifrons* larvae differs across host species. Breeding season activity and size differ between *Neoconocephalus* katydids in Kentucky, and therefore some of these species may be more suitable for parasitoid development. I predicted that *N. triops* would be a suitable host for successful development of *Ormia lineifrons* pupae. In addition, the clutch size can affect developmental success. For example, larger clutch sizes reduce the developmental success of the larvae inside the host. Thus, I predicted that larger clutch sizes in *Neoconocephalus* hosts will reduce the proportion of successfully developing *O. lineifrons* flies. To answer these questions of parasitoid development, I measured the clutch sizes among four hosts: *Neoconocephalus triops*, *Neoconocephalus velox*,

Neoconocephalus robustus, Neoconocephalus nebrascensis. Further, I measured the development time of *Ormia lineifrons* pupae, the mass of pupae, and the developmental success of *Ormia lineifrons* pupae across host species.

I found that *Ormia lineifrons* successfully developed in four different *Neoconocephalus* species. Additionally, *Ormia lineifrons* larvae had a significantly higher success rate when using *N. velox* as a host, compared to *N. triops.* The clutch size did not differ among *Neoconocephalus* hosts. Furthermore, I found no difference in pupal development time among the offspring emerging from different hosts. Pupal mass decreased the most when the clutch size went from one to two. Further, *N. robustus* and *N. triops* had heavier pupae than *N. velox*. *Ormia lineifrons* exerts selective pressure across all these species throughout their reproductive season in Kentucky. Unexpectedly, the observed clutch size of *O. lineifrons* was smaller than the optimal clutch size. The host usage and development of *O. lineifrons* are discussed.

Introduction

Parasites can affect their hosts in multiple ways, e.g., by altering their behavior, morphology, or physiology (see Krist 1999; Dingemanse et al. 2009; Hoang et al. 2017; Timi & Poulin 2020). Crickets of the genus *Nemobius* show altered behavior when parasitized by the hairworm, *Paragordius tricuspidatus*, by jumping into water for the parasite to complete its lifecycle and killing itself consequently (Sanchez et al. 2008). Fisher (1963) showed that caterpillars parasitized with the ichneumonid *Nemeritis canescens* elicited a physiological change in response to parasitism. The success of the *Nemeritis* larvae was higher with higher levels of available oxygen, but the host

caterpillar showed a physiological suppression of the parasites through asphyxiation. Parasitic interactions affecting their respective host can be a primer that results in a co-evolutionary arms race between the hosts and parasites, promoting adaptive diversity. This diversity is evident through new adaptations arising to better resist parasitism or bypass host responses.

Eavesdropping acoustic parasitoids are remarkable at exploiting host calls (Edgecomb et al. 1995; Robert et al. 1992; Robert 2001; Robert & Göpfert 2002; Akcakaya & Nehorai 2008; Arthur & Hoy 2006). This is evident for example in that these parasitoids have adapted their hearing sensitivity to match the call frequency of their hosts, facilitating host detection (e.g., Robert et al. 1992). However, it is similarly important to consider developmental capabilities of the parasitoid within a host. Because after the female parasitoid has located a host and deposited her mobile planidial larvae, the focus shifts to her offspring's development within a host and ability to overcome any hosts immune responses and conspecific competition. Examining the clutch size, offspring success rates, development time, and progeny size (i.e., mass, length, width) are important factors to consider when studying parasitoid development (Godfray 1994; Lehmann 2003).

The clutch size that yields the maximum fitness return is known as the Lack clutch size (Lack 1947; review in Godfray 1994). However, the observed clutch size in field populations is frequently smaller than the optimal clutch size (Hardy et al. 1992; Vet et al. 1994). Smaller observed clutch sizes may be the result of the parasitoid's inability to assess host quality (Adamo et al. 1995; Allen et al. 1999), changes in the searching costs associated with host prevalence (Allen 1995), or a limitation of available female

eggs (Waage et al. 1985). The effects of female parasitoid clutch size have been explored in many systems (e.g., Waage et al. 1985; Zaviezo & Mills 2000). Female parasitoids are thought to be capable of adjusting their clutch size when parasitizing different hosts, as an inability to do so, would affect the success rate of her progeny (Godfray 1994). There may be variability in clutch sizes when there are differences in host quality related to host size (Hardy et al. 1992; Zaviezo & Mills 2000). Larger hosts are thought to be higher quality hosts because of an expected increase in progeny success rate due to increased access to host resources (Harvey et al. 1994; Lehmann 2003; Lehmann 2008).

Good quality hosts lead to higher success rates and thus inclusive fitness of the parasitoid (e.g., Colinet et al. 2005). Host usage can differ between species because hosts can differ in size (Lehmann 2008), immune susceptibility (Adamo et al. 1995), life histories (Whitesell 1974), and/or calling and breeding season activity (SINA 2020).

Parasitoid developmental times are typically uniform as host sizes differ (Carpenter et al. 1994). However, the parasitoid *Cotesia glomerata* has a shorter development time in larger hosts (e.g., *Pieris brassicae)* compared to smaller hosts (e.g., *Pieris rapae;* Harvey 2000), whereas in some instances, longer development times are associated with larger hosts because parasitoid's take longer to consume the host (Sandlan 1982). In some parasitoids, larger clutch sizes reduce offspring developmental success (e.g., Allen 1995; Welch 2006) and shortens offspring development time (Bouletreau 1971). The shortened development time may be the result of the larvae experiencing contest competition and therefore emerge from the host sooner (see Allen & Hunt 2001). The shorter developmental time may also benefit the parasitoid because the larvae would become reproductively active sooner (Hunt & Allen 2000).

Flies in the family Tachinidae are eavesdropping endoparasitoids of insects (Stireman III et al. 2006). Recent progress has been made characterizing some parasitoid systems within *Ormiini*, many others still require comprehensive study (e.g., Burk 1982; Adamo et al. 1995; Welch 2006; Gray et al. 2007; Lehmann 2008). The eavesdropping parasitoid, *O. lineifrons*, parasitizes *Neoconocephalus triops* (Burk 1982) and *Orchelimum nigripes* (Shapiro 1995). Burk (1982) and I have previously determined that *N. triops* suffers high levels of parasitism by *O. lineifrons* (Lehmann 2003; Chapter II). Besides *N. triops*, I found that *O. lineifrons* also uses *N. velox*, *N. robustus* and *N. nebrascensis* as hosts, at different times in the year in Kentucky. Thus, *O. lineifrons* uses and successfully develops in multiple host species in Kentucky (Chapter I).

My study aims to understand if and how *O. lineifrons* larval development varies among different *Neoconocephalus* hosts and different clutch sizes. Determining what affects *O. lineifrons* development will be essential for understanding how efficient this parasitoid utilizes multiple host systems. In this study, I examined the effect of host species and clutch size on pupal mass, pupal developmental time to adult fly, and success rate of development. In addition, I measured the size of each host species sampled. I hypothesized that developmental success, developmental time, and mass of pupae differ with increasing clutch sizes and among host species. Specifically, I predicted that larger clutch sizes increase competition and would lead to lower pupal mass, shorter development time, and lower success rates of *O. lineifrons* larvae. Additionally, I predicted that larger host species increase pupal mass and developmental success of the larvae. However, it is difficult to predict how the developmental time is affected by the host size based on the mixed results in the literature and it could either be lengthened or

shortened in bigger hosts. This is the first study of *O. lineifrons* development in *Neoconocephalus* hosts.

Materials and Methods

Collection of Neoconocephalus katydids

I collected katydids within a 30-mile radius around Murray, KY between March, and October in 2019 and 2020. I did not collect females for this project since it is the males that attract the flies. After collection by hand, I placed a katydid in a centrifuge vial (50mL; Falcon brand) for transport to the lab. In the lab, each katydid was placed in a single cage (15.57 x 23.19 x 15.25 cm, height x length x width) with ad libitum organic apple slices, cricket food (Fluker's High-calcium cricket diet) and rolled oats for food. I supplied animals ad libitum with water gel (Tasty Worms Nutrition, Inc.) and sprayed cages daily with water. Every two days, I replaced the apple slices, and added water gel and rolled oats as needed. I glued a mesh screen to the underside of the perforated cage lid to prevent parasitoid larvae from escaping. I kept the cages in an incubator (PR505755L; Thermo Fisher Scientific) with a light/dark cycle of 15.5/8.5h and coinciding temperatures of 26/22ºC (day/night). I maintained a relative humidity of 65 - 85% in the incubator for all katydids and pupae.

Parasitism

I checked daily each cage for the presence of fly larvae, fly pupae, and katydid mortality. I thoroughly checked food and water gel containers for the presence of larvae and pupae because the larvae will roam in the cage to find a place to pupate (KJR pers. obs.). Dead katydids were kept in the incubator for 24 hours to allow time for any

remaining larvae inside the host to emerge. I then dissected the thorax and abdomen of the katydid under a dissecting scope (S6-RLT; Richter Optica) and checked for larvae inside the dead host. *Ormia lineifrons* larvae typically emerge from the katydid within nine days (Burk 1982). I kept unparasitized *Neoconocephalus* in the lab for at least six weeks before I froze (- 20ºC) them. The hind right femur of these dead katydids was measured.

Pupae care

I placed pupae in the centrifuge vials on the same day they emerged from a host. I placed the pupa at the base of the tube (50mL; Falcon brand) on cotton that was soaked with distilled water. I checked that the breathing funnels of the pupae were not blocked with water or debris. The lids of the centrifuge tubes had holes for gas exchange and the tubes were placed upright in the same incubator as the katydids (temperatures & day lengths see above). I checked the centrifuge vial daily for bacterial/fungal growth on the pupa and cotton, as well as cotton dryness. I replaced the cotton plug when bacterial/fungal growth was detected and sprayed the cotton with water when necessary. In 2019 I placed each emerged adult fly in a communal cage and preserved it in 99% ethanol after death. In 2020 I euthanized flies by freezing (- 20ºC for 24 hours). I preserved all fly specimens, but some of the specimens from 2019 were severely dried out and did not preserve well. The flies were identified as *O. lineifrons* by Dr. James O'Hara (Chapter II). In 2019, I placed all pupa that emerged from a host in the same centrifuge tube. However, in 2020, I placed each pupa in a separate centrifuge tube, which provided a more standardized environment that excluded potential pupal interactions. In my analyses, this difference in handling is coded for as a 'year' effect.

Ethics statement

My animal care and experimental procedures adhered to the ASAB/ABS guidelines for the use of animals in research, the legal requirements of the U.S.A., and all guidelines of Murray State University.

Morphological measurements & developmental success rate

I measured several morphological features of the hosts and fly pupae within +/- 0.01 mm using digimatic calipers (Mitutoyo; Seiko Instruments Inc.). For hosts, I measured the hind right femur length of dead males as the host size indicator (see Lehmann & Lehmann 2006).

For each pupa, I measured the mass (Ohaus brand; Model PA84) of each pupa on the day of emergence from the host. I measured the fly development success rate, the pupal developmental time, and the clutch size for each host. To determine fly development success rate, I calculated the ratio of pupa to hatching adult flies for each host. The developmental time was determined by counting the number of days between pupa emergence from the host and when the pupa hatched as an adult fly. Lastly, the clutch size was counted as the number of pupae that emerged and any remaining larvae inside the dead host.

Statistical Analysis

I analyzed whether development success rate, development time, and pupa mass differed among hosts species, clutch sizes, and years. Linear models for both developmental time and pupa mass used the factors ‘year’, ‘clutch size’, and ‘host species’ and all two-way and three-way interactions as fixed effects. The logistic regression model for success rate (i.e., clutch size successfully developing larvae per

host) used the factors ‘year’ and ‘host species’ along with all two-way interactions as fixed effects.

I compared the success rate of *O. lineifrons* pupa among species and between years by using a Chi-square test. The pupa development time was tested using a likelihood-ratio test to test if development time differed among ‘host species’, ‘clutch size’, or ‘years’. I used an ANOVA to assess how pupal mass was affected by ‘host species’, ‘years’, ‘clutch size’, and ‘clutch size squared’. The clutch size squared term was used to account for the non-linear trend in the model. I compared the host size among the four *Neoconocephalus* species using an ANOVA. I used an ANOVA to compare the mean clutch size among ‘host species’, ‘years’, and the interaction of ‘species x year’. I removed all non-significant interactions stepwise from all models and I present the reduced models in the Results. I used post-hoc Tukey-Kramer tests to determine significant pairwise differences. I used the software JMP (version 15.2.1 for Mac) to run the ANOVAs and R (Version 1.4.1106) to run the logistic regression models.

Results

The development success rate of *O. lineifrons* pupae was significantly higher in *N. velox* than in *N. triops* (Tukey HSD: $p = 0.02$) and was higher in 2020 than in 2019 (likelihood-ratio test: $\chi^2 = 6.58$, DF = 1, $p = 0.01$). Pupal success rates were 50% in *N. triops*, 75% in *N. velox*, 85% in *N. robustus*, and 57% in *N. nebrascensis* (**Figure 2-1**). *Ormia lineifrons* pupae were successful 73% of the time in 2020 and only 52% in 2019 across host species (**Figure 2-2**). Pupal success rate was not affected by clutch size and ranged between 20% - 88% (**Figure 2-3**).

The mean clutch size of *O. lineifrons* did not differ among hosts (ANOVA: $F_{3,79} = 1.87$, $p = 0.14$, **Table 2-1**) or between years (ANOVA: $F_{1,79} = 0.03$, $p = 0.87$). Likewise, the mean development time of *O. lineifrons* pupae did not differ among hosts (likelihood-ratio test: $\chi^2 = 0.26$, DF = 3, $p = 0.86$), clutch size (likelihood-ratio test: $\chi^2 = 1.09$, DF = 1, $p = 0.30$), or between years (likelihood-ratio test: $\chi^2 = 0.35$, DF = 1, $p = 0.56$, **Table 2-1**).

The mean pupae mass decreased as clutch size increased (likelihood-ratio test: $\chi^2 = 36.85$, DF = 1, $p < 0.0001$). The rate of change decreased as clutch size increased, i.e., it was largest for the decrease from a clutch size of one to two and much less from clutches of two to four (**Figure 2-4**). The mean pupae mass was significantly different among hosts (ANOVA: $F_{3,79} = 6.44$, $p = 0.00009$), with *N. robustus* and *N. triops* having significantly heavier *O. lineifrons* pupae than *N. velox* (Tukey HSD: $p = 0.001$ for both comparisons, **Figure 2-5**). The mean pupal mass of *N. nebrascensis* was not different from *N. triops* and *N. robustus*. Furthermore, the mean pupae mass was heavier in 2020 than in 2019 (likelihood-ratio test: $\chi^2 = 5.76$, DF = 1, $p = 0.02$, **Figure 2-6**).

There were significant differences in host size (ANOVA: $F_{3,105} = 94.60$, $p = 0.0001$, **Figure 2-7**). Specifically, *N. robustus* was larger than all other species (Tukey HSD: $p = 0.0001$ for each comparison) and *N. nebrascensis* was larger than *N. velox* and *N. triops* (Tukey HSD: $p = 0.0001$ for *N. triops*, $p = 0.002$ for comparison with *N. velox*). There was no difference in the mean size of *N. velox* and *N. triops* males (Tukey HSD: $p = 0.76$).

Discussion

In my comparison of larval development of *O. lineifrons* among four katydid hosts, I found both differences and similarities across hosts. I found no difference in the

mean clutch size that *O. lineifrons* placed on each host nor in the developmental times of the pupae. *Ormia lineifrons* larvae had higher success rates when developing in *N. velox* than *N. triops*, even though these species were the same size. Fly pupal mass was significantly heavier in *N. robustus* and *N. triops* compared to *N. velox.* Across species, pupal mass decreased significantly with clutch size.

Clutch size & Competition

Clutch size greatly influences gregarious parasitoid development (Charnov & Skinner 1984; Waage et al. 1985). The realized clutch size (from field) is often smaller than the optimal clutch size a parasitoid can allocate to a host (Kolluru & Zuk 2001). This discrepancy between observed and optimal clutch sizes may be the result of a negative relationship between clutch size and the fitness of adult flies (Kolluru & Zuk 2001). I found the same mean clutch size across species and a lower mean field clutch size (2 larvae) than the optimal (3-4 larvae). The discrepancy seen between the Lack clutch size and the realized clutch size may be due to competitive trade-offs among the larvae or higher fitness returns for the parasitoid. Parasitoid larvae experience more exploitative competition in larger clutch sizes, and subsequently develop into smaller adults (Vet et al. 1994). Larger adult females are more fecund in hymenopteran (Godfray 1994) and tachinid parasitoids (i.e., Nakamura 1995). Therefore, it is beneficial to maintain smaller clutch sizes because they yield bigger females and thus higher fitness returns for the parasitoid (Kolluru & Zuk 2001).

Gregarious parasitoids, such as *O. lineifrons*, typically experience competition primarily through resource exploitation of the host (Brodeur & Boivin 2004). Host resources can be divided equally (scramble) or unequally (contest) leading to different

levels of competition experienced by the parasitoid larvae (Lehmann 2008). I predicted that larger clutch sizes would reduce the mean pupal mass and success rate. The rate at which *O. lineifrons'* pupal mass decreased, was largest between clutch sizes of one to two, whereas the change between two to three and three to four were less pronounced (**Figure 2-4**). *Ormia lineifrons* showed similar success rates (~60%) for clutch sizes up to four and much lower success rates when the clutch size was five (~20%, **Figure 2-3**). The gregarious parasitoid *Homotrixa alleni* had survivorship that remained constant with clutch sizes up to five and then sharply decreased (Allen & Hunt 2001). Both *O. lineifrons* and *H. alleni* are gregarious tachinids that show similar survivorship patterns as a function of clutch size. It was suggested that *H. alleni* experienced scramble competition in smaller clutch sizes, but when the clutch size reached (or exceeded) a threshold, the larvae experienced contest competition (Allen & Hunt 2001). Consequently, *O. lineifrons* may experience similar competitive interactions that inhibit the success of larger clutch sizes.

Reproductive strategies & Host usage

I predicted that larger host species would produce heavier pupae. I found that *O. lineifrons* pupae were heaviest in the two largest host species, *N. robustus* and *N. nebrascensis* (**Figure 2-5**, **Figure 2-7**), supporting my prediction. Interestingly, *O. lineifrons* pupae were heavier in *N. triops* than in *N. velox,* despite these species being the same size (**Figure 2-5**, **Figure 2-7**). The mass discrepancy observed between *N. triops* and *N. velox* pupae may be due to differences between the species' life history. Male *N. triops* overwinter as adults in temperate habitats such as Kentucky, whereas other temperate *Neoconocephalus* species (i.e., *N. velox*) overwinter in the egg stage (Whitesell

1974). This may lead to fundamental differences in fat reserves available for *O. lineifrons* larvae to exploit in each host. *Neoconocephalus triops* overwinters as adult and typically has large quantities of fat in preparation for diapause (Whitesell 1974). In contrast, summer *N. triops* lack conspicuous amounts of fat (Whitesell 1974). However, it is possible that the fat reserves are mostly used up by the time the overwintering *N. triops* start singing in Kentucky, possibly providing less nutrients to the developing larvae compared to the non-diapausing *N. velox* males.

The literature provided contradictory directions how host size would affect development time of the larvae and it was difficult to propose a prediction. I found that *O. lineifrons* took the same amount of time (~12 days, **Table 2-1**) to develop in large and small hosts. The constancy of developmental time across different host sizes may suggest that there has been strong selection on this duration. For example, longer development could increase the risk of the host being superparasitized, increasing larval competition for resources inside the host. Additionally, longer development also increases the chances of the host being preyed upon or dying of other circumstances, which would directly affect the larvae as well. Development times shorter than twelve days may lead to smaller adult females that have reduced fecundity (Nakamura 1995). Considering that the mean clutch size across species was around two, the constant developmental time may have evolved based on competition among two larvae across host species. However, more research is needed to test this, and other hypotheses related to the developmental time of *O. lineifrons*.

Ormia lineifrons successfully uses four different *Neoconocephalus* hosts for its larvae. It did not adjust its clutch size nor development time based on differences in the

size of the host species nor the success of larval development, suggesting these traits may present a compromise that allows to *O. lineifrons* to utilize multiple hosts rather than one. The latter is especially important, because *O. lineifrons* has three generations per year and needs to use a different host for each generation. The poor success of larval development in the first host species, *N. triops*, may restrict the population size of subsequent *O. lineifrons* generations, suggesting that selection on improving the usage of *N. triops* might be strong and underway.

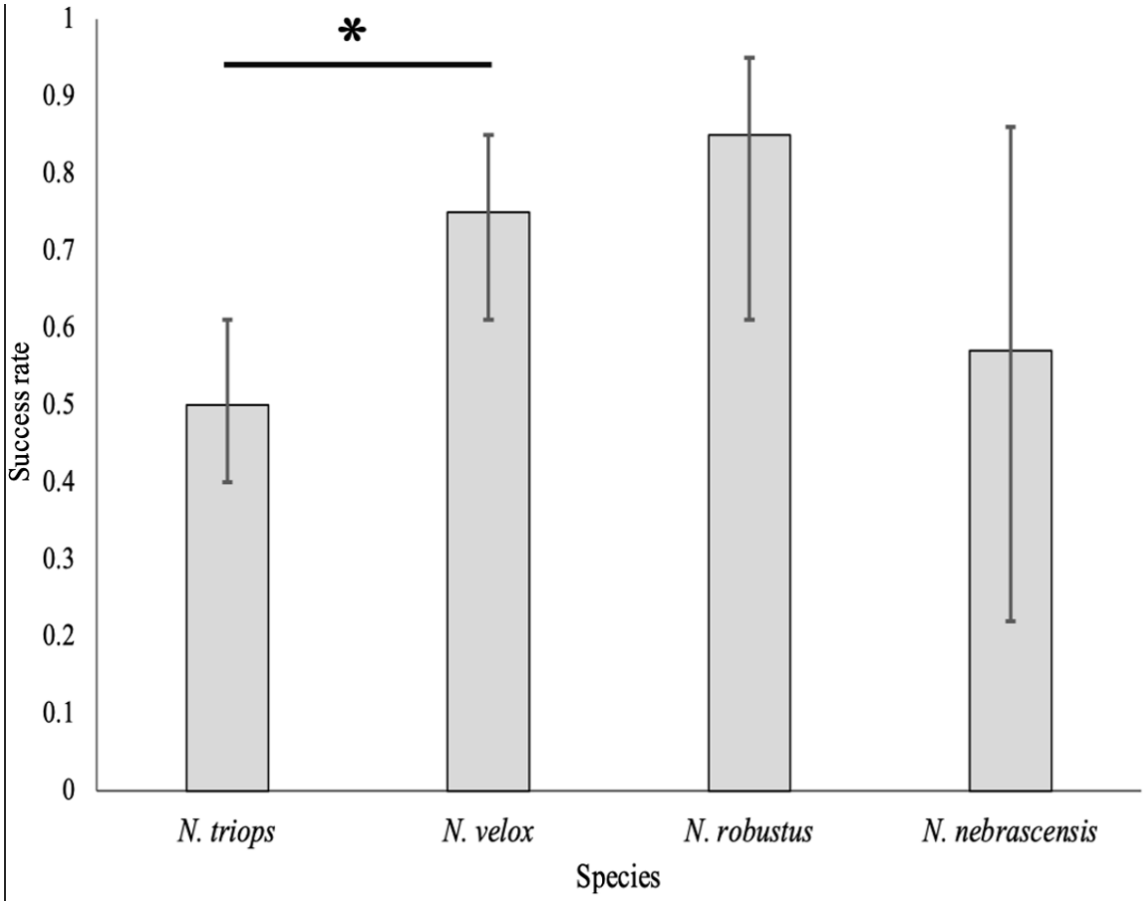

Figure 2-1: The predicted probabilities of *O. lineifrons* success rate among host species are shown as percentages with 95% confidence intervals. The confidence intervals are not symmetric because those percentages are based on an inverse logit transformation of the log odds-ratio from the logistic regression. Asterisks denote significant differences in the predicted probabilities of success rate.

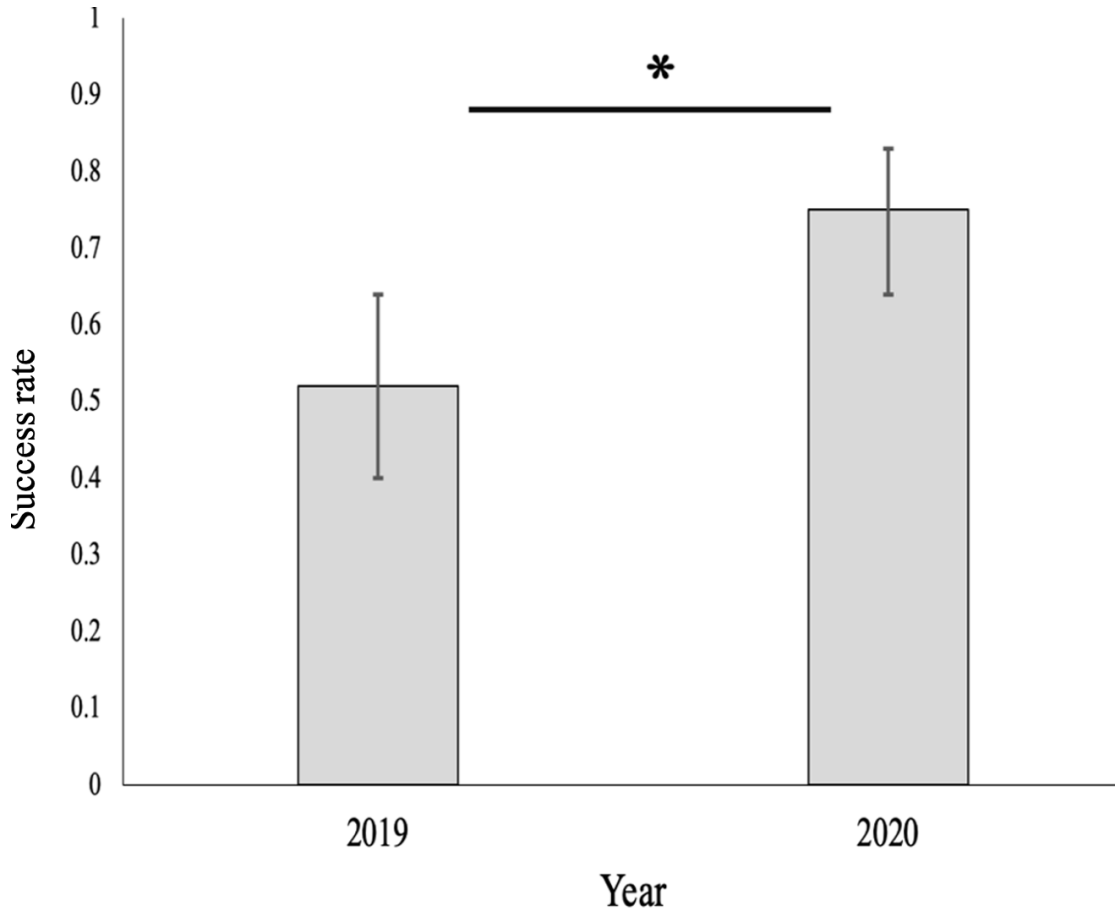

Figure 2-2: The predicted probabilities of *O. lineifrons* success rate between 2019 and 2020 shown as percentages with 95% confidence intervals. The confidence intervals are not symmetric because those percentages are based on an inverse logit transformation of the log odds-ratio from the logistic regression. Asterisks denote significant differences in the predicted probabilities of success rate.

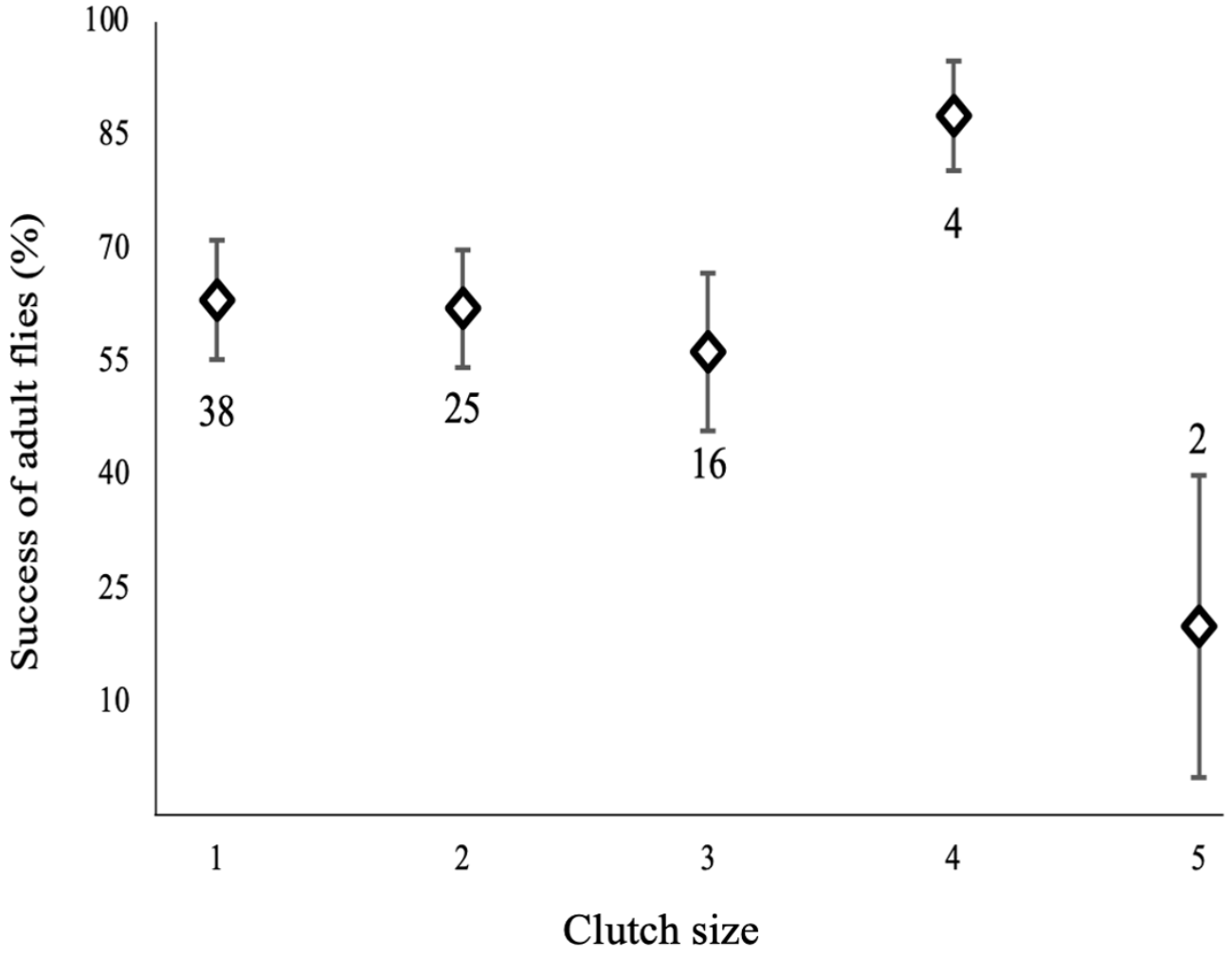

Figure 2-3: The mean success rate (±SE) of *O. lineifrons* pupae as a function of clutch size. The numbers below the data points represent sample sizes (N).

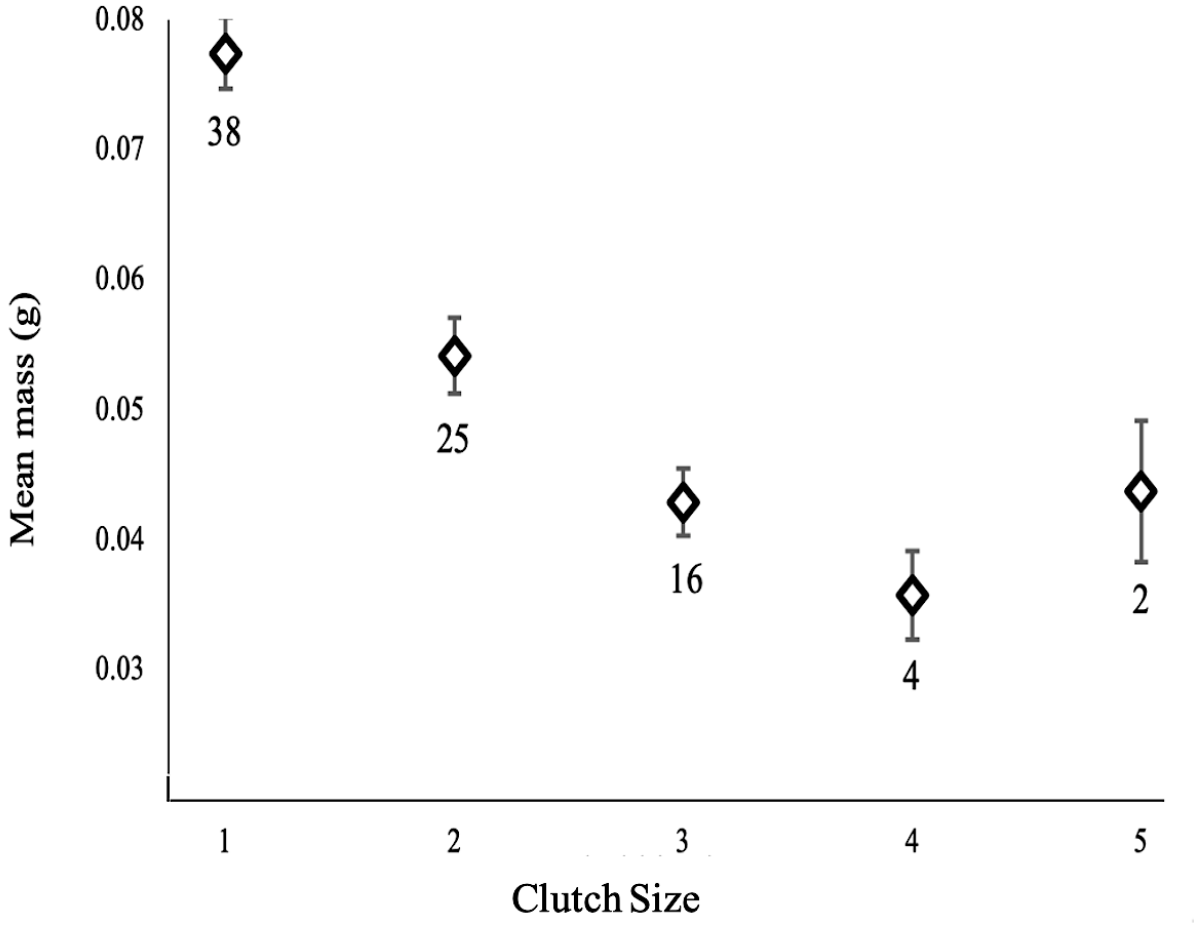

Figure 2-4: The mean mass (±SE) of *O. lineifrons* pupae as a function of clutch size. Numbers below the data points represent sample sizes (N).

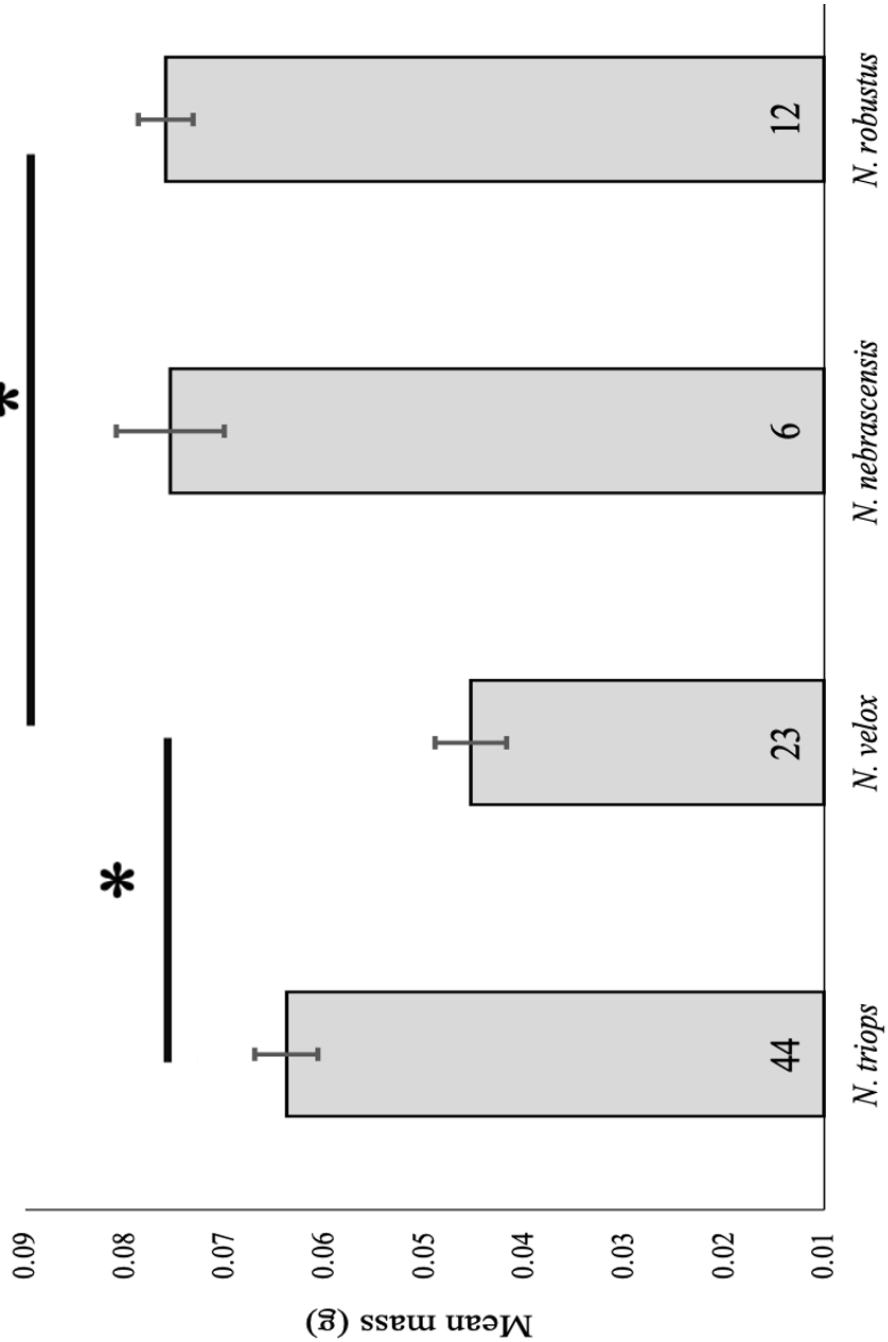

Figure 2-5: The mean mass (±SE) of *O. lineifrons* pupae among host species. Asterisks denote significant differences in mean mass among the hosts. Sample sizes (N) are indicated in each column.

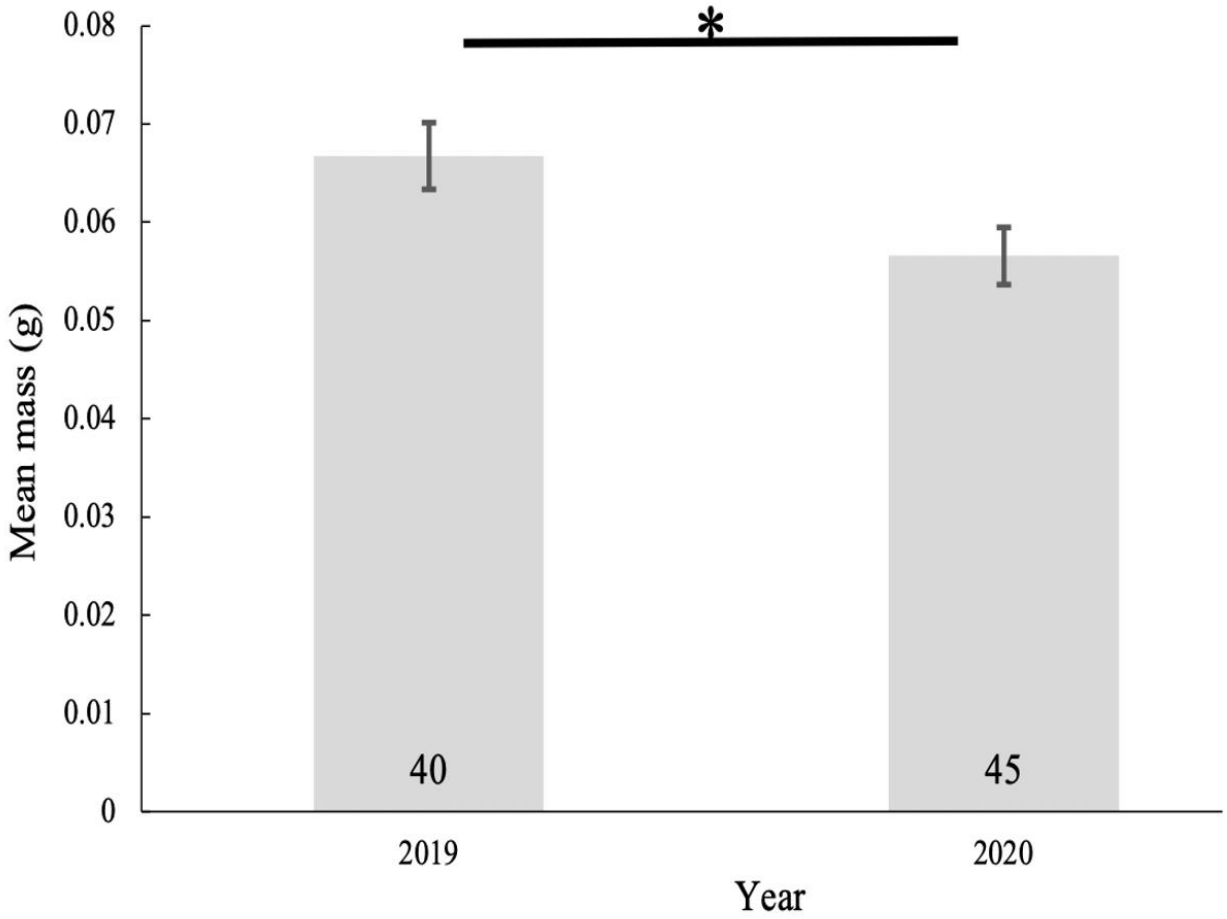

Figure 2-6: The mean mass (±SE) of *O. lineifrons* pupae between 2019 and 2020. Asterisks denote significant differences in mean mass. Numbers at the base of each column are the sample sizes (N).

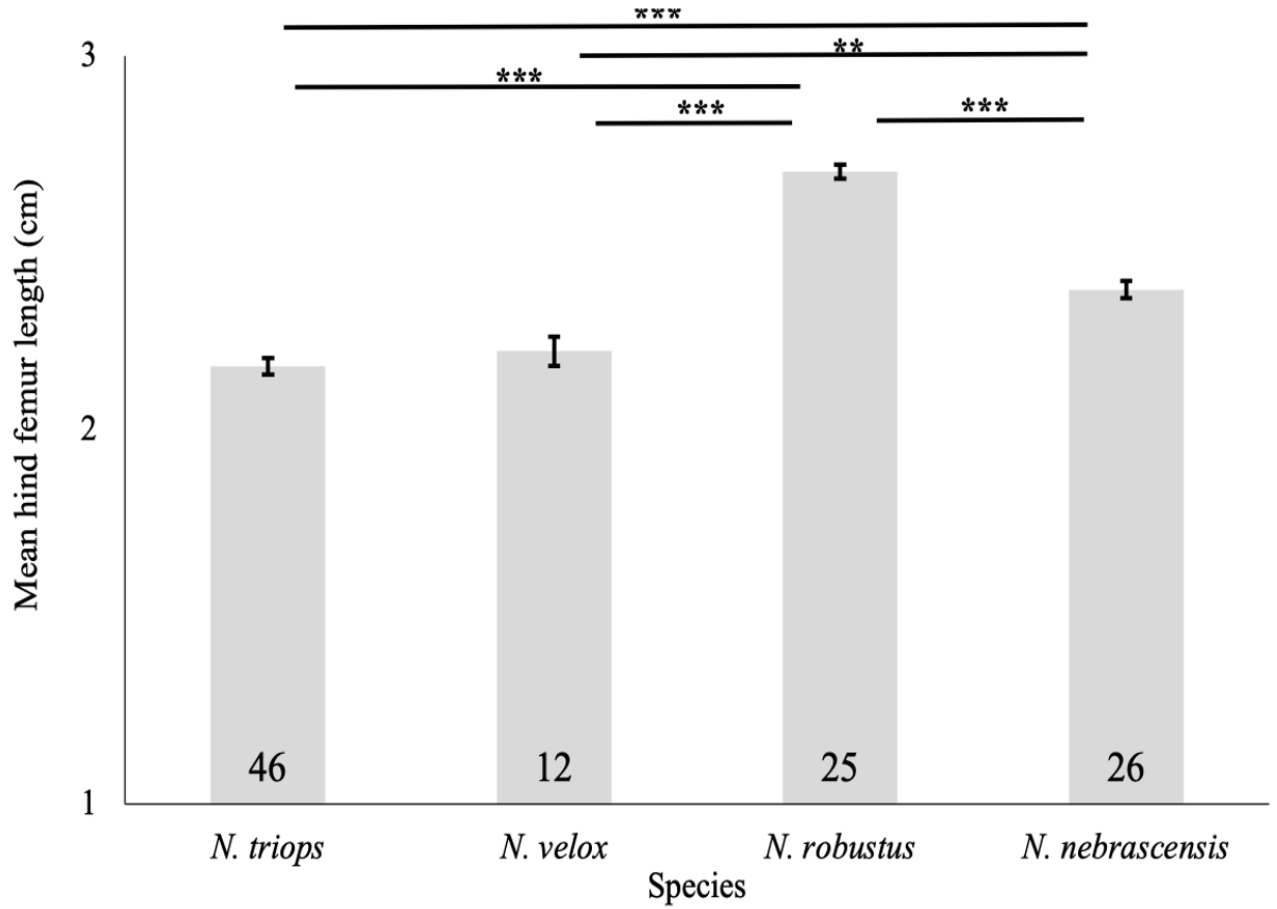

Figure 2-7: The mean (±SE) hind right femur length as an indicator of size for the host species. Numbers in each column represent sample size (N). Asterisks represent significant size differences.

Table 2-1: The mean clutch size (±SD) among the different *Neoconocephalus* hosts and the mean development time (±SD) of *O. lineifrons* larvae. Sample sizes (N) are represented in parenthesis.

Species	Mean development time (days±SD)	Mean clutch size (±SD)
N. triops	12.33±1.1 (N=25)	1.93±0.97 (N=44)
N. velox	12.51±3.0 (N=21)	2.30±1.18 (N=23)
N. robustus	12.10±1.0 (N=10)	1.42±0.67 (N=12)
N. nebrascensis	12.50±1.9 (N=4)	1.17±0.41(N=6)

DISCUSSION

This study adds multiple important findings to the current understanding of *Ormia lineifrons* and its host usage In Kentucky, I found that *O. lineifrons* has three distinct generations, each parasitizing different *Neoconocephalus* species throughout those reproductive seasons. The three different generations are likely the result of *O. lineifrons'* life cycle, wherein the timing of each generation coincides with different katydid hosts because of host synchronization. Both *N. triops* and *N. velox* are active when no other *Neoconocephalus* species is active, and these two species face extraordinary rates of parasitism as their seasons progresses. Similarly, populations of *Sciarasaga quadrata* (Allen 1995; Hunt & Allen 2000), *Poecilimon mariannae* (Lehmann 2008), and *Gryllus sp.* (Cade 1975; Gray et al. 2007) show increased rates of parasitism with season progression. Eventually, *O. lineifrons* kills all the *N. triops* and *N. velox* males by the end of their season. The third generation of *O. lineifrons* parasitizes *N. robustus* and *N. nebrascensis.* However, these species experience lower rates of parasitism compared to *N. triops* and *N. velox.*

Ormia lineifrons has the capability to successfully develop in at least four *Neoconocephalus* hosts. Additionally, I found that larvae had significantly higher developmental success rates in *N. velox* than in *N. triops*. Moreover, *N. velox* had significantly smaller pupae than *N. robustus* and *N. triops*. Interestingly, the success rate of *O. lineifrons* larvae was not affected by clutch size (i.e., same success rate in different

clutch sizes). I did not find any differences in the mean clutch size or larvae development time among host species.

Parasites and parasitoids can exert selective pressures on their hosts through altering their behavior, morphology, or physiology (Krist 1999; Dingemanse et al. 2009; Hoang et al. 2017; Timi & Poulin 2020). Thus, the interactions and behaviors present in these systems are an integral component for generating diversity through these arms races. Both studies (CHAPTER I & CHAPTER II) add invaluable information to the *Ormia lineifrons* and *Neoconocephalus* katydid systems and more general to the understanding of the selective pressures at work shaping both the parasitoid as well as the host species.

LITERATURE CITED

Adamo, S. A., Robert, D., & Hoy, R. R. (1995). Effects of a tachinid parasitoid, *Ormia ochracea*, on the behaviour and reproduction of its male and female field cricket hosts (*Gryllus spp*). *Journal of Insect Physiology*, *41*(3), 269-277.

Akcakaya, M., & Nehorai, A. (2008). Performance analysis of the Ormia ochracea's coupled ears. *The Journal of the Acoustical Society of America*, *124*(4), 2100-2105.

Allan, R. A., Elgar, M. A., & Capon, R. J. (1996). Exploitation of an ant chemical alarm signal by the zodariid spider Habronestes bradleyi Walckenaer. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *263*(1366), 69-73.

Allen, G. R. (1995). The calling behaviour and spatial distribution of male bushcrickets (Sciarasage quadrata) and their relationship to parasitism by acoustically orienting tachinid flies. *Ecological Entomology*, *20*(4), 3030-310.

Allen, G. R. (2000). Call structure variability and field survival among bushcrickets exposed to phonotactic parasitoids. *Ethology*, *106*(5), 409-423.

Allen, G. R., & Hunt, J. (2001). Larval competition, adult fitness, and reproductive strategies in the acoustically orienting ormiine Homotrixa alleni (Diptera: Tachinidae). *Journal of insect Behavior*, *14*(3), 283-297.

Allen, G. R., Kamien, D., Berry, O., Byrne, P., & Hunt, J. (1999). Larviposition, host cues, and planidial behavior in the sound-locating parasitoid fly Homotrixa alleni (Diptera: Tachinidae). *Journal of Insect Behavior*, *12*(1), 67-79.

Alphen, J. V., & van Vet, L. E. M. (1986). An evolutionary approach to host finding and selection In: Insect Parasitoids, Waage J., Greathead, D.

Alphen, J. V., & Visser, M. E. (1990). Superparasitism as an adaptive strategy for insect parasitoids. *Annual review of entomology*, *35*(1), 59-79.

Arthur, B. J., & Hoy, R. R. (2006). The ability of the parasitoid fly *Ormia ochracea* to distinguish sounds in the vertical plane. *The Journal of the Acoustical Society of America*, *120*(3), 1546-1549.

Askew, R. R., & Shaw, M. R. (1986). Parasitoid Communities: Their Size, Structure and Development. 225–264. *Waage, J and D Greathead, D.(eds.)*.

Beckers, O. M., & Schul, J. (2008). Developmental plasticity of mating calls enables acoustic communication in diverse environments. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1640), 1243-1248.

Beckers, O. M., & Schul, J. (2010). Female adaptation to developmental plasticity in male calling behavior. *Behavioral ecology and sociobiology*, *64*(8), 1279-1290.

Beckers, O. M., & Wagner Jr, W. E. (2011). Male field crickets infested by parasitoid flies express phenotypes that may benefit the parasitoids. *Animal Behaviour*, *82*(5), 1151-1157.

Beckers, O. M., & Wagner Jr, W. E. (2018). Males and females evolve riskier traits in populations with eavesdropping parasitoids. *Behavioral Ecology and Sociobiology*, *72*(11), 1-11.

Beckers, O. M., Martin, C. M., & Wagner Jr, W. E. (2011). Survival rates of planidial larvae of the parasitoid fly Ormia ochracea (Diptera: Tachinidae). *Journal of the Kansas Entomological Society*, *84*(3), 235-237.

Beckers, O. M., Murphey, K. J., Pease, J. R., & Norman, N. (2019). Parallel plasticity of mating songs and preferences in the field cricket Gryllus rubens. *Ethology*, *125*(7), 476-484.

Belwood, J. J., & Morris, G. K. (1987). Bat predation and its influence on calling behavior in Neotropical katydids. *Science*, *238*(4823), 64-67.

Bernal, X. E., Rand, A. S., & Ryan, M. J. (2006). Acoustic preferences and localization performance of blood-sucking flies (*Corethrella Coquillett*) to túngara frog calls. *Behavioral Ecology*, *17*(5), 709-715.

Bertram, S. M., Xochitl Orozco, S., & Bellani, R. (2004). Temporal shifts in conspicuousness: mate attraction displays of the Texas field cricket, *Gryllus texensis*. *Ethology*, *110*(12), 963-975.

Biron, D. G., Ponton, F., Marché, L., Galeotti, N., Renault, L., Demey-Thomas, E., ... & Thomas, F. (2006). 'Suicide' of crickets harbouring hairworms: a proteomics investigation. *Insect molecular biology*, *15*(6), 731-742.

Bouletreau, M. (1971). Larval growth and host consumption in Pteromalus puparum (Hymenoptera Chalcidoidea): effect of population density. In *Annales de zoologie: Ecologie animale*.

Bradbury, J. W., & Vehrencamp, S. L. (2011). Principles of animal communication, 2nd edn Sunderland. *MA: Sinauer Associates*.

Bretman, A., & Tregenza, T. (2007). Strong, silent types: the rapid, adaptive disappearance of a sexual signal. *Trends in ecology & evolution*, *22*(5), 226-228.

Brodeur, J., & Boivin, G. (2004). Functional ecology of immature parasitoids. *Annual Reviews in Entomology*, *49*(1), 27-49.

Burk, T. (1982). Evolutionary significance of predation on sexually signaling males. *The Florida Entomologist*, *65*(1), 90-104.

Burk, T. (1988). Acoustic signals, arms races and the costs of honest signalling. *Florida Entomologist*, 400-409.

Büttner, U. K. (2002). Charakterisierung der Gesänge von fünf in Missouri (USA) heimischen *Neoconocephalu*s-Arten (Orthoptera, Tettigoniidae). Diploma thesis, University of Erlangen, Germany.

Cade, W. (1975). Acoustically orienting parasitoids: fly phonotaxis to cricket song. *Science*, *190*(4221), 1312-1313.

Cade, W. H. (1984). Effects of fly parasitoids on nightly calling duration in field crickets. *Canadian Journal of Zoology*, *62*(2), 226-228.

Cade, W. H., Ciceran, M., & Murray, A. M. (1996). Temporal patterns of parasitoid fly (Ormia ochracea) attraction to field cricket song (Gryllus integer). *Canadian Journal of Zoology*, *74*(2), 393-395.

Calero-Torralbo, M. A., & Valera, F. (2008). Synchronization of host-parasite cycles by means of diapause: host influence and parasite response to involuntary host shifting. *Parasitology*, *135*(11), 1343.

Carpenter, J. E., Pair, S. D., & Fitt, G. P. (1994). Ichneumon promissorius (Hymenoptera: Ichneumonidae): development on North American hosts. *Journal of economic entomology*, *87*(4), 929-932.

Charnov, E. L., & Skinner, S. W. (1984). Evolution of host selection and clutch size in parasitoid wasps. *Florida Entomologist*, 5-21.

Checklist of Katydids North of Mexico. Katydids North of Mexico. (n.d.). https://orthsoc.org/sina/katylist.htm.

Civantos, E., Ahnesjö, J., & Forsman, A. (2005). Immune function, parasitization and extended phenotypes in colour polymorphic pygmy grasshoppers. *Biological Journal of the Linnean Society*, *85*(3), 373-383.

Colinet, H., Salin, C., Boivin, G., & Hance, T. H. (2005). Host age and fitness-related traits in a koinobiont aphid parasitoid. *Ecological Entomology*, *30*(4), 473-479.

Crosskey, R. W. (1965). The immature stages and affinities of the tachinid fly *Glaurocara flava*, a parasite of the African bush-cricket *Homorocoryphus nitidulus vicinus*. In *Proceedings of the Zoological Society of London*, *144*(2), 203-218. Oxford, UK: Blackwell Publishing Ltd.

Darwin, C. (1859). The origin of species and the descent of man, New York (The Modern Library).

de Silva, P., Jaramillo, C., & Bernal, X. E. (2014). Feeding site selection by frog-biting midges (Diptera: Corethrellidae) on anuran hosts. *Journal of insect behavior*, *27*(3), 302-316.

Dingemanse, N. J., Oosterhof, C., Van Der Plas, F., & Barber, I. (2009). Variation in stickleback head morphology associated with parasite infection. *Biological Journal of the Linnean Society*, *96*(4), 759-768.

Edgecomb, R. S., Robert, D., Read, M. P., & Hoy, R. R. (1995). The tympanal hearing organ of a fly: phylogenetic analysis of its morphological origins. *Cell and tissue research*, *282*(2), 251-268.

Feener Jr, D. H., & Brown, B. V. (1997). Diptera as parasitoids. *Annual review of entomology*, *42*(1), 73-97.

Fisher, R. C. (1963). Oxygen requirements and the physiological suppression of supernumerary insect parasitoids. *Journal of Experimental Biology*, *40*(3), 531-540.

Gerhardt, H. C., & Huber, F. (2002). *Acoustic communication in insects and anurans: common problems and diverse solutions*. University of Chicago Press.

Godfray, H. C. J., & Godfray, H. C. J. (1994). *Parasitoids: behavioral and evolutionary ecology* (Vol. 67). Princeton University Press.

Gray, D. A., & Cade, W. H. (1999). Sex, death, and genetic variation: natural and sexual selection on cricket song. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *266*(1420), 707-709.

Gray, D. A., Banuelos, C., Walker, S. E., Cade, W. H., & Zuk, M. (2007). Behavioral specialization among populations of the acoustically orienting parasitoid fly *Ormia ochracea* utilizing different cricket species as hosts. *Animal Behaviour*, *73*(1), 99-104.

Hardy, I. C. W., Griffiths, N. T., & Godfray, H. C. J. (1992). Clutch size in a parasitoid wasp: a manipulation experiment. *Journal of Animal Ecology*, 121-129.

Harvey, J. A. (2000). Dynamic effects of parasitism by an endoparasitoid wasp on the development of two host species: implications for host quality and parasitoid fitness. *Ecological Entomology*, *25*(3), 267-278.

Harvey, J. A., Harvey, I. F., & Thompson, D. J. (1994). Flexible larval growth allows use of a range of host sizes by a parasitoid wasp. *Ecology*, *75*(5), 1420-1428.

Harris, V. E., & Todd, J. W. (1980). Male-mediated aggregation of male, female, and 5th-instar southern green stink bugs and concomitant attraction of a tachinid parasite, *Trichopoda pennipes*. *Entomologia Experimentalis et applicata*, *27*(2), 117-126.

Hoang, K., Tao, L., Hunter, M. D., & de Roode, J. C. (2017). Host diet affects the morphology of monarch butterfly parasites. *Journal of Parasitology*, *103*(3), 228-236.

Huigens, M. E., Pashalidou, F. G., Qian, M. H., Bukovinszky, T., Smid, H. M., van Loon, J. J., ... & Fatouros, N. E. (2009). Hitch-hiking parasitic wasp learns to exploit butterfly antiaphrodisiac. *Proceedings of the National Academy of Sciences*, *106*(3), 820-825.

Hunt, J., & Allen, G. R. (2000). Larval density and developmental instability in the acoustically orienting parasitoid Homotrixa alleni. *acta ethologica*, *2*(2), 129-132.

Kolluru, G. R., & Zuk, M. (2001). Parasitism patterns and the size-fecundity relationship in the acoustically orienting dipteran parasitoid Ormia ochracea. *Canadian Journal of Zoology*, *79*(6), 973-979.

Kotiaho, J., Alatalo, R. V., Mappes, J., & Parri, S. (1996). Sexual selection in a wolf spider: male drumming activity, body size, and viability. *Evolution*, *50*(5), 1977-1981.

Krist, A. C. (1999). The effects of parasites and predators on the life history and morphology of freshwater snails.

Lack, D. (1947). The significance of clutch-size. *Ibis*, *89*(2), 302-352.

Lehmann, G. U. (2003). Review of biogeography, host range and evolution of acoustic hunting in Ormiini (Insecta, Diptera, Tachinidae), parasitoids of night-calling

bushcrickets and crickets (Insecta, Orthoptera, Ensifera). *Zoologischer Anzeiger-A Journal of Comparative Zoology*, *242*(2), 107-120.

Lehmann, G. U. (2008). How different host species influence parasitism patterns and larval competition of acoustically orienting parasitoid flies (Tachinidae: Ormiini). *Animal Behavior: New Research*, 93-132.

Lehmann, G. U., & Heller, K. G. (1998). Bushcricket song structure and predation by the acoustically orienting parasitoid fly Therobia leonidei (Diptera: Tachinidae: Ormiini). *Behavioral Ecology and Sociobiology*, *43*(4), 239-245.

Lehmann, G. U., & Lehmann, A. W. (2006). Potential lifetime reproductive success of male bushcrickets parasitized by a phonotactic fly. *Animal Behaviour*, *71*(5), 1103-1110.

Lehmann, G. U., Heller, K. G., & Lehmann, A. W. (2001). Male bushcrickets favored by parasitoid flies when acoustically more attractive for conspecific females (Orthoptera: Phanopteridae/Diptera: Tachinidae). *Entomologia Generalis*, *25*(2), 135-140.

Léonide, J., & Léonide, J. C. (1969). Insectes parasites et predateurs des Diptères acridiophages; parasites occasionnels des Orthoptéres en Provence. *Bulletin de la Société entomologique de France*, *74*(1), 21-32.

Lewkiewicz, D. A., Zuk, M. (2004). Latency to Resume Calling after Disturbance in the Field Cricket, *Teleogryllus oceanicus*, Corresponds to Population-Level Differences in Parasitism Risk. *Behavioral Ecology and Sociobiology, 55*(6), 569-573.

Local Weather Forecast, News and Conditions. Weather Underground. (n.d.). http://www.wunderground.com/.

Mangold, J. R. (1978). Attraction of *Euphasiopteryx ochracea*, *Corethrella sp*. and *gryllids* to broadcast songs of the southern mole cricket. *Florida Entomologist*, 57-61.

McGregor, P. K. (Ed.). (2005). *Animal communication networks*. Cambridge University Press.

Nakamura, S. (1995). Optimal clutch size for maximizing reproductive success in a parasitoid fly, Exorista japonica (Diptera: Tachinidae). *Applied Entomology and Zoology*, *30*(3), 425-431.

Nutting, W. L. (1953). The biology of *Euphasiopteryx brevicornis* (Townsend) (Diptera, Tachinidae), parasitic in the cone-headed grasshoppers (Orthoptera, Copiphorinae). *Psyche*, *60*(2), 69-81.

Paur, J., & Gray, D. (2011). Seasonal dynamics and overwintering strategy of the tachinid fly (Diptera: Tachinidae), *Ormia ochracea* (Bigot) in southern California. *Terrestrial Arthropod Reviews*, *4*(2), 145-156.

Robert, D. (2001). Innovative biomechanics for directional hearing in small flies. *The Biological Bulletin*, *200*(2), 190-194.

Robert, D., & Göpfert, M. C. (2002). Novel schemes for hearing and orientation in insects. *Current opinion in neurobiology*, *12*(6), 715-720.

Robert, D., Amoroso, J., & Hoy, R. R. (1992). The evolutionary convergence of hearing in a parasitoid fly and its cricket host. *Science*, *258*(5085), 1135-1137.

Ryan, M. J., & Keddy-Hector, A. (1992). Directional patterns of female mate choice and the role of sensory biases. *The American Naturalist, 139*, S4-S35.

Sabrosky, C. W. (1953). Taxonomy and host relations of the tribe *Ormiini i*n the Western Hemisphere (Diptera, Larvaevoridae*). Proceedings of the Entomological Society of Washington,* 55, 167–183.

Sanchez, M. I., Ponton, F., Schmidt-Rhaesa, A., Hughes, D. P., Misse, D., & Thomas, F. (2008). Two steps to suicide in crickets harboring hairworms. *Animal Behaviour*, *76*(5), 1621-1624.

Sandlan, K. P. (1982). Host suitability and its effects on parasitoid biology in Coccygomimus turionellae (Hymenoptera: Ichneumonidae). *Annals of the Entomological Society of America*, *75*(3), 217-221.

Schul, J., Patterson, A. C. (2003). What determines the tuning of hearing organs and the frequency of calls? A comparative study in the katydid genus *Neoconocephalus* (Orthoptera; Tettigoniidae). *Journal of Experimental Biology,* 206, 141–152.

Shapiro, L. (1995). Parasitism of Orchelimum katydids (Orthoptera: Tettigoniidae) by Ormia lineifrons (Diptera: Tachinidae). *Florida Entomologist*, 615-615.

Simmons, L. W., Zuk, M., & Rotenberry, J. T. (2001). Geographic variation in female preference functions and male songs of the field cricket *Teleogryllus oceanicus*. *Evolution*, *55*(7), 1386-1394.

Stireman III, J. O., O'Hara, J. E., & Wood, D. M. (2006). Tachinidae: evolution, behavior, and ecology. *Annu. Rev. Entomol.*, *51*, 525-555.

Timi, J. T., & Poulin, R. (2020). Why ignoring parasites in fish ecology is a mistake. *International Journal for Parasitology*, *50*, 755-761.

Tinghitella, R. M., & Zuk, M. (2009). Asymmetric mating preferences accommodated the rapid evolutionary loss of a sexual signal. *Evolution: International Journal of Organic Evolution*, *63*(8), 2087-2098.

Vélez, M. J., & Brockmann, H. J. (2006). Seasonal variation in selection on male calling song in the field cricket, *Gryllus rubens*. *Animal Behaviour*, *72*(2), 439-448.

Vet, L. E., Datema, A., Janssen, A., & Snellen, H. (1994). Clutch size in a larval-pupal endoparasitoid: consequences for fitness. *Journal of Animal Ecology*, 807-815.

Vincent, C. M., & Bertram, S. M. (2010). Crickets groom to avoid lethal parasitoids. *Animal Behaviour*, *79*(1), 51-56.

Vinson, S. B., & Iwantsch, G. F. (1980). Host regulation by insect parasitoids. *The Quarterly Review of Biology*, *55*(2), 143-165.

Waage, J. K., Hassell, M. P., & Godfray, H. C. J. (1985). The dynamics of pest-parasitoid-insecticide interactions. *Journal of Applied Ecology*, 825-838.

Wagner Jr, W. E. (1996). Convergent song preferences between female field crickets and acoustically orienting parasitoid flies. *Behavioral Ecology*, *7*(3), 279-285.

Wagner Jr, W. E. (2011). Direct benefits and the evolution of female mating preferences: conceptual problems, potential solutions, and a field cricket. *Advances in the Study of Behavior*, *43*, 273-319.

Wagner Jr, W. E., & Reiser, M. G. (2000). The importance of calling song and courtship song in female mate choice in the variable field cricket. *Animal behaviour*, *59*(6), 1219-1226.

Walker, T. J. (1993). Phonotaxis in female *Ormia ochracea* (Diptera: Tachinidae), a parasitoid of field crickets. *Journal of insect behavior*, *6*(3), 389-410.

Walker, T. J., & Wineriter, S. A. (1991). Hosts of a phonotactic parasitoid and levels of parasitism (Diptera: Tachinidae: *Ormia ochracea*). *Florida Entomologist*, 554-559.

Walker, T. J., Whitesell, J. J., Alexander, R. D. (1973). The robust conehead: two widespread sibling species (Orthoptera: Tettigoniidae: *Neoconocephalus* '*robustus'*). *Ohio Journal of Science*, *73*(6), 321–330.

Welch, C. H. (2006). Intraspecific competition for resources by *Ormia depleta* (Diptera: Tachinidae) larvae. *Florida Entomologist*, *89*(4), 497-501.

Whitesell, J. J. (1974). *Geographic Variation and Dimorphisms in Song, Development, and Color in a Katydid; Field and Laboratory Studies (*Tettigoniidae, Orthoptera*)* (Doctoral dissertation, University of Florida).

Whitesell, J. J., & Walker, T. J. (1978). Photoperiodically determined dimorphic calling songs in a katydid. *Nature*, *274*(5674), 887-888.

Wineriter, S. A., & Walker, T. J. (1990). Rearing phonotactic parasitoid flies [Diptera: Tachinidae, *ormiini, ormia spp*.]. *Entomophaga*, *35*(4), 621-632.

Wyatt, T. D. (2014). *Pheromones and animal behavior: chemical signals and signatures*. Cambridge University Press.

Zaviezo, T., & Mills, N. (2000). Factors influencing the evolution of clutch size in a gregarious insect parasitoid. *Journal of Animal Ecology*, *69*(6), 1047-1057.

Zuk, M., & Kolluru, G. R. (1998). Exploitation of sexual signals by predators and parasitoids. *The Quarterly Review of Biology*, *73*(4), 415-438.

Zuk, M., Rotenberry, J. T., & Simmons, L. W. (1998). Calling songs of field crickets (*Teleogryllus oceanicus*) with and without phonotactic parasitoid infection. *Evolution*, *52*(1), 166-171.

Zuk, M., Rotenberry, J. T., & Tinghitella, R. M. (2006). Silent night: adaptive disappearance of a sexual signal in a parasitized population of field crickets. *Biology letters*, *2*(4), 521-524.

Zuk, M., Simmons, L. W., & Cupp, L. (1993). Calling characteristics of parasitized and unparasitized populations of the field cricket *Teleogryllus oceanicus*. *Behavioral Ecology and Sociobiology*, *33*(5), 339-343.

Zuk, M., Simmons, L. W., & Rotenberry, J. T. (1995). Acoustically orienting parasitoids in calling and silent males of the field cricket *Teleogryllus oceanicus*. *Ecological Entomology*, *20*(4), 380-383.