COURTSHIP ACOUSTICS AND MATING IN *Cotesia*, A GENUS OF PARASITOID WASPS

A Dissertation

by

ANDREA LEE JOYCE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2007

Major Subject: Entomology

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Approved by:

Co-Chairs of Committee,	Julio Bernal
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Committee Members,	Robert Wharton
	Jane Packard
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ABSTRACT

Courtship Acoustics and Mating in *Cotesia*, A Genus of Parasitoid Wasps.

(August 2007)

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Cotesia are parasitoid wasps (Hymenoptera: Braconidae) that are used for biological control of pest moths (Lepidoptera: Noctuidae, Pyralidae) that damage agricultural crops. This dissertation investigated courtship acoustics and mating, and their relevance to biological control, in members of the *Cotesia flavipes* species complex, and a noncomplex member, *Cotesia marginiventris*.

The first study investigated whether courtship acoustics were species specific for two members of the *Cotesia flavipes* complex, *C. flavipes* and *C. sesamiae*, and for *C. marginiventris.* During courtship, male *Cotesia* fan their wings and produce low amplitude sounds and substrate vibrations. The airborne and substrate components of courtship were similar within a species. However, the courtship acoustics of each species was distinct. The duration and frequency of several courtship acoustic components distinguished each species, while some components did not differ among species.

The second study investigated mating success and transmission of courtship vibrations on natural and artificial rearing substrates for *Cotesia marginiventris*. Mating success was measured on plastic, glass, corn and bean leaves, and chiffon fabric. Mating success was lowest on plastic and glass, intermediate on corn and bean leaves, and highest on chiffon. Substrate influenced transmission of courtship vibrations. Durations of courtship vibrations were longer on corn, bean and chiffon than on plastic. Frequency modulation occurred on corn, bean and chiffon, and amplitude was greatest on chiffon. The mating success of normal and dealated males was higher on chiffon than on glass, suggesting that courtship communication relied in part on substrate vibrations.

The third study examined female and male mate choice in a solitary and a gregarious species, *C. marginiventris* and *C. flavipes*, respectively. Females of the solitary species, *C. marginiventris,* mated more frequently with large than small males, and this did not appear to be the result of male competition. Male choice for female size was not apparent in *C. marginiventris*. Females of the gregarious parasitoid, *C. flavipes*, mated with large or small males with similar frequencies, and male-male competition was not observed. In the male choice experiment, *C. flavipes* males attempted copulation and mated more with smaller females, and smaller females accepted males more than large females.

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Dr. Jim Whitfield and Dr. Jonathan Banks at University of Illinois, Urbana-Champaign, checked the species identity of *C. flavipes* and *C. sesamiae* using mitochondrial COI. Dr. John Westbrook and Paul Schleider at U.S.D.A., A.R.S., in College Station, Texas loaned an ultrasonic detector and shared expertise to listen for ultrasonic signals from the wasps. Dr. Richard Mankin and Dr. John Sivinski at U.S.D.A., A.R.S., Gainesville, Florida, confirmed that I was properly measuring courtship acoustic signals.

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CHAPTER I

INTRODUCTION

Parasitoid wasps are insects in which the larva develops entirely in or on another insect and consumes the host insect. They are used frequently in biological control programs to reduce populations of economically important pest insects. The goal of my dissertation research was to assess the importance of courtship acoustics in *Cotesia* Cameron (Hymenoptera: Braconidae), a genus of parasitoid wasps, with emphasis on the relevance of those acoustics to biological control. This introduction summarizes the literature on acoustic courtship signaling in parasitoids.

Males and females of many parasitoid wasp species exhibit specific courtship behaviors prior to mating. Male parasitoid wasps generally court females, and courtship can include chemical and acoustic signals (van den Assem, 1986). Acoustic courtship signals include both airborne and substrate vibrations. Previous studies of parasitoid courtship acoustics characterized the signals and the information they might convey, such as species identity. Only a few studies demonstrated that parasitoid wasps responded to airborne or substrate vibrations produced during courtship. However, in other behavioral contexts, such as host finding, parasitoid wasps detected and responded to vibrational cues, which suggests that they are capable of detecting the airborne and/or substrate vibrational cues produced during courtship.

This dissertation follows the format of *Journal of Insect Behavior*.

Distinguishing Airborne and Substrate Vibration

Courtship acoustics include both airborne and substrate vibrations. A vibrating object creates waves that travel through air, liquid, or solid substrates. Waves compress and rarify (relax) the medium through which they travel (Tauber and Eberl, 2003), and consist of both a pressure component, and a particle velocity component, from motion of molecules induced by the wave. There are at least three ways by which insects detect or 'hear' airborne or substrate vibration (Ewing, 1989; Gerhardt and Huber, 2002; Greenfield, 2002). The first is airborne sound, or far-field sound, where pressure receptors, such as the tympanum on a grasshopper, detect pressure waves (Gerhard and Huber, 2002). Hymenoptera are not known to perceive far-field sound (Greenfield, 2002). Secondly, insects may detect near-field sound produced by particle velocity near the source of vibration, and it is typically detected within several cm of the vibrating source (Eliopoulos, 2006). Among Hymenoptera, near-field sound is used in the dance communication of honeybees (Towne and Kirchner, 1989), and detected by a Johnston's organ in their antennae (Dreller and Kirchner, 1993). Finally, vibrations can be transmitted through the substrate and detected by insects through the subgenual organ in the tibia (Čokl, 1983). Substrate vibrations are thought to be perceived by the subgenual organ in Hymenoptera, including ants, bees and parasitoids, as they are perceived by other insects (McIndoo, 1922; Menzel and Tautz, 1994; Vilhelmsen *et al.,* 2001).

Parasitoid Acoustic Courtship Signals

During courtship, male parasitoids produce sounds and vibrations associated with wing fanning, leg tapping, rocking, and other behaviors. Parasitoids from many families exhibit male wing fanning prior to copulation with females, and in some species after copulation as well. These families include Ichneumonidae, Braconidae, Aphelinidae, Chalcididae, Eulophidae, Pteromalidae, and Mymaridae (Vinson, 1972; Kitano, 1975; Weseloh, 1977; Gordh and DeBach, 1978; Leonard and Ringo, 1978; Vinson, 1978; van den Assem and Putters, 1980; Tagawa and Kitano, 1981; Wharton, 1984; Field and Keller, 1993a; Ruther *et al.,* 2000). In Encyrtidae and Scelionidae, courtship acoustics are produced only after mounting and may include wing fanning. The Trichogrammatidae do not appear to engage in wing fanning, and male-female interactions may be mediated by chemical or visual stimuli (Gordh and DeBach, 1978), suggesting a decrease in the use of vibrational signals for more derived lineages of parasitoids.

Male wing fanning during courtship may play multiple roles, and several hypotheses for the function of wing fanning have been proposed, including: 1) wing fanning helps males orient to female pheromone (Vinson, 1972, 1978); 2) wing fanning is acoustic communication between males and females (Sivinski and Webb, 1989; Field and Keller, 1993a; van den Assem and Putters, 1980), and; 3) wing fanning may push a male pheromone toward a female (Ruther *et al.,* 2000). Independently of these hypotheses, female pheromones generally appear to induce male wing fanning during courtship (Ruther *et al.,* 2000).

Acoustic courtship patterns are unique for a number of parasitoid species, and may provide information about species identity. Courtship sounds were recorded using a microphone and described for *Cotesia marginiventris* (Cresson), *Diachasmimorpha kraussii* Fullaway*, D. longicaudata* (Ashmead), and *Microplitis croceipes* (Cresson) (all Braconidae)*,* and sound patterns were distinct for each species (Sivinski and Webb, 1989; Rungrojwanich and Walter, 2000). Gordh and DeBach (1978) used the mean number of precoital wing vibration pulses to distinguish *Aphytis* Howard species groups in the family Aphelinidae. Several species of Pteromalidae were found to have unique courtship sounds generated by wing movements (van den Assem and Putters, 1980), and a microphone and phonograph cartridge recorded similar patterns of airborne and substrate vibrations. Given their specificity, acoustic signals could be a diagnostic tool for distinguishing closely related species of parasitoid wasps within a genus, or to compare populations or strains within a species, as they have been useful for discriminating cryptic species in other insects, such as *Chrysoperla carnea* (Stephens) and *Enchenopa binotata* (Say) (Henry, 1994; Lin and Wood, 2002).

Wing fanning by males during courtship appears to be essential for mating success in some parasitoids, but not in others (Ruther *et al.,* 2000). Mating success in dealated male *Nasonia vitripennis* (Walker) was reduced from ~70% to 20%, and older dealated males were less likely to mate than younger males (Miller and Tsao, 1974; van den Assem and Putters, 1980), suggesting that wings and/or courtship acoustics produced by wing fanning were important for mating success. In separate studies, wing ablation lowered the mating success of male *Cotesia glomerata* (L.) and *D. kraussii*, in the later case from ~80% to 20%, though in another study 90% of wingless *C. glomerata* mated (Kitano, 1975; Tagawa and Hidaka, 1982; Rungrojwanich and Walter, 2000). The variation between these studies has not been explained, but may be related to differences in the physiological condition of the organisms, substrates on which matings occurred, or wing ablation procedures, among other factors.

Some evidence exists that parasitoid wasps detect the near-field sounds or substrate vibrations produced by male wing fanning during courtship. Female *D. longicaudata* responded to male courtship sounds that were replayed as audible, airborne sounds into an arena, and females were significantly more active than males (Sivinski and Webb, 1989). Since Hymenoptera are not known to detect far-field sound, this suggests that the parasitoids responded to either near-field sound, or substrate vibrations, from the audible replay. Field and Keller (1993a) studied mating in *Cotesia rubecula* (Marshall), and reported a courtship song containing a low frequency sound and pulsing, apparently transmitted through the substrate. Pulsing by males on leaves shared with females induced female receptivity, indicated by a drop antennae behavior in females. Females on leaves not shared with courting males did not exhibit the drop antennae behavior to signal receptivity. The study by Field and Keller (1993a) is the clearest evidence to date that parasitoids respond to courtship substrate vibrations.

Courtship acoustics additionally may be used by parasitoid females to assess male quality. Young male *N. vitripennis* with wings glued to the thorax had higher mating success than older males with glued wings, while with sound replayed both young and old males had similar mating rates (van den Assem and Putters, 1980). This suggested that acoustic courtship signals may convey information about male quality. The method used to replay the signals in that study was not described, therefore it is unclear whether wasps responded to near-field sound or substrate vibration.

Parasitoid Host Location Using Vibrations

Much of the work addressing parasitoid detection of substrate vibrations has been conducted in the behavioral context of host finding, in which parasitoid wasps respond to substrate vibrations produced by their hosts. Meyhöfer and Casas (1999) provided a thorough review of parasitoid host finding using vibratory stimuli. The parasitoid *Sympiesis sericeiornis* Nees (Eulophidae), which parasitizes the leaf miner *Phyllonorycter malella* (Ger.) (Gracillaridae), responded to host-produced vibrations while searching for hosts. Using the concealed larvae of the stemboring Mexican Rice Borer (*Eoreuma loftini* Dyar), Tomov *et al.* (2003) showed that *Parallorhogas pyralophagas* (Marsh) (Braconidae) drilled and oviposited less frequently in larvae whose activity levels were reduced after feeding on host plant tissue containing GNA, a protease inhibiting lectin. Sokolowski and Turlings (1987) using a temperature sensitive strain of *Drosophila melanogaster* Meigen showed that the parasitoid *Asobara tabida* Nees (Braconidae) more frequently probed active hosts than inactive hosts. Parasitoids also use self-produced vibrations to find hidden hosts, a process known as vibrational sounding. Wasps that drummed their antennae on a substrate produced vibrations (Henaut, 1990; Wackers *et al.,* 1998), which were thought to be received by the subgenual organ in the tibia (Vilhelmsen *et al.,* 2001). Parasitoid wasps that use this mechanism typically attack stem boring, wood boring, or soil dwelling hosts (Broad and Quicke, 2000; Vilhelmsen *et al.,* 2001).

Clearly, parasitoid wasps and other Hymenoptera respond to substrate vibration and near-field sound in various behavioral contexts including host location, and have specialized morphological structures for their detection. Thus, parasitoid wasps may be able to detect and respond to near-field sounds or substrate vibrations produced by mates during courtship.

Cotesia **Parasitoid Wasps**

The focus in this dissertation is several species of *Cotesia*, parasitoid wasps in the family Braconidae (Hymenoptera), subfamily Microgastrinae (Wharton *et al.,* 1997). Braconidae is one of the parasitoid families most widely used for biological control of pest insects (Wharton, 1993). Typically, they are either ectoparasitoids or endoparasitoids, and females oviposit either one or a clutch of eggs within or on the larval host. The endoparasitoids typically exhibit koinobiosis, where hosts continue to develop for a time before a parasitoid larva emerges from the host to pupate. Upon emergence from pupae, adults search for mates and subsequently host larvae for oviposition. The genus *Cotesia* has a world-wide distribution, and species are solitary or gregarious parasitoids (Wharton, 1993). At least 70 *Cotesia* spp. are recorded in North America north of Mexico, and there are an estimated 1000 species world-wide (Mason, 1981; Whitfield, 1995; Whitfield, 1997). The studies in this dissertation included gregarious species that are members of the *Cotesia flavipes* Cameron complex, and one solitary species, *C. marginiventris*, all used previously for biological control of various Lepidoptera pest species.

The members of the *Cotesia flavipes* species complex, which include *C. flavipes*, *C. sesamiae* (Cameron), and *C. chilonis* (Matsumura), have been used for biological control for stem boring pests, such as *Chilo partellus* (Swinhoe) and *Diatraea saccharalis* (F.) (Polaszek and Walker, 1991; Potting, 1996; Overholt *et al.,* 1997), which attack graminaceous crops, such as corn, sorghum, and sugarcane (Polaszek and Walker, 1991; Smith *et al.,* 1993; Potting, 1996). *Cotesia flavipes* is distributed throughout the Indo-Australian region, *C. sesamiae* is found in Africa, and *C. chilonis* has been collected from Japan and China (Kimani-Njogu *et al.,* 1997). Two of the species, *C. chilonis* and *C. sesamiae*, are exceptionally difficult to distinguish using morphological characters. Distinguishing between these species in Africa is important because *C. sesamiae* is native to Africa, while *C. chilonis* and *C. flavipes* were introduced as biological control agents (Overholt *et al.,* 1997). Host range, morphology, host finding, and molecular studies have been used to separate these species and some strains (Hailemichael *et al.,* 1997; Kimani-Njogu and Overholt, 1997; Kimani-Njogu *et al.,* 1997; Sallam *et al.,* 1999; Smith and Kambhampati, 1999; Mochiah *et al.,* 2001; Mochiah *et al.,* 2002; Michel-Salzat and Whitfield, 2004; Muirhead *et al.,* 2006). For example, the strain of *C. sesamiae* from eastern Kenya, which can not develop in *Busseola fusca* (Fuller), is genetically distinct from *C. sesamiae* from western Kenya, which develops in *B. fusca* (Mochiah *et al.,* 2001; Muirhead *et al.,* 2006).

Cotesia marginiventris is considered an important natural control agent for several agricultural pests, including *Plathypena scabra* (F.)*, Rachiplusia nu* (Guenée), *Spodoptera exigua* (Hübner)*, S. frugiperda* (J. E. Smith), and *Trichoplusia ni* (Hübner) (all Noctuidae) (McCutcheon and Turnipseed, 1981; Braman and Yeargan, 1991; Novoa and Luna, 1996; Gillespie *et al.*, 1997). *Cotesia marginiventris* is a solitary endoparasitoid that oviposits in first or second instar larvae, only one parasitoid adult emerges from each larval host (Boling and Pitre, 1970; Kunnalaca and Mueller, 1979; Tillman 2001), and is distributed throughout the Americas (Marsh, 1979). Its reproductive biology has been studied (Braman and Yeargan, 1991; Riggin *et al.*, 1992; Tillman, 2001; Riddick, 2002), and it has been considered for augmentative biological control and tested in greenhouses for control of *T. ni* on peppers and cucumbers (Gillespie *et al.*, 1997; Urbaneja *et al.*, 2002). The following studies were undertaken in this dissertation to investigate courtship acoustics and mate choice for *Cotesia* parasitoids:

Study 1: Can Courtship Acoustics Separate Closely Related Species of Parasitoids Within the* Cotesia flavipes *Complex?

This study focused on members of the *Cotesia flavipes* complex. The goal was to compare and determine if the courtship acoustics were unique for three closely related species, *C. flavipes*, *C. sesamiae,* and *C. chilonis,* and a *Cotesia* species that is not part of the complex, *Cotesia marginiventris*. *Cotesia chilonis* was not available, so comparisons were limited to *C. flavipes*, *C. sesamiae*, and the non-complex member, *C.*

marginiventris. The courtship acoustics were expected to be unique for each species. In addition, different populations of *C. sesamiae* were recorded, from western and eastern Kenya, with differential abilities to develop in *B. fusca*. The courtship acoustics pattern for strains of *C. sesamiae* were expected to be more similar to each other than to those from another species.

Study 2: Investigate the Role of Substrate on Mating, Courtship Vibration Transmission, and Acoustic Communication for **C. marginiventris**

Mating in arthropods that use vibrational communication is influenced by substrate. If *C. marginiventris* relies on substrate vibrations for courtship communication, female mating success may be influenced by rearing substrates. Hence, determining whether *C. marginiventris* uses airborne and/or substrate courtship signals for courtship communication, and whether rearing substrates affect the transmission of these signals, could improve mating success, and therefore improve rearing of this parasitoid for augmentative biological control.

Study 3: Mate Choice for a Solitary and Gregarious **Cotesia** ***Species***

This study compared mate choice in a solitary parasitoid, *Cotesia marginiventris*, and a gregarious parasitoid, *Cotesia flavipes*. Mate choice could be influenced by the mating structure of the parasitoid (Godfray, 1994; Godfray and Cook, 1997). In this study, the question was addressed whether female or male mate choice of mating partners occurred for *C. marginiventris* and *C. flavipes*, and whether mate choice depended on the outcome of male-male competition. Airborne sounds play a role in mate choice in many insects (Gerhardt and Huber, 2002), but mate choice in insects using vibrational signals has rarely been addressed. The potential role of vibrational signals in mate choice was addressed.

CHAPTER II

COURTSHIP ACOUSTICS OF THE *C. flavipes* COMPLEX

Introduction

Correct identification of parasitoid insects is essential to biological control, but accurate identification of species based on morphology alone can be challenging. *Cotesia* Cameron species are parasitoids in the family Braconidae, and are used in biological control of Lepidoptera pests (Wharton, 1993). *Cotesia* species are either solitary, producing one offspring per host, or gregarious, with several progeny emerging from each larval host (Mason, 1981). The *Cotesia flavipes* complex is comprised of three species, *C. flavipes* Cameron, *C. sesamiae* (Cameron), and *C. chilonis* (Matsumura). These species have been used extensively for biological control of economically important stem boring insects, such as *Chilo partellus* (Swinhoe) and *Diatraea saccharalis* (F.), in more than 40 countries (Polaszek and Walker, 1991; Overholt *et al*., 1997; Potting *et al*., 1997). *Cotesia flavipes* is native to the Indo-Australian region, *C. sesamiae* is indigenous in Africa, and *C. chilonis* occurs in Japan and China (Kimani-Njogu and Overholt, 1997). *Cotesia chilonis* and *C. flavipes* were introduced into Africa for biological control, so discriminating these species and the native parasitoid *C. sesamiae* is important (Overholt *et al.,* 1997). Additionally, it is possible that cryptic species exist within the complex, as suggested by differences between populations of *C. flavipes* from Southeast Asia and Australia (Polaszek and Walker, 1991).

Two of the species, *C. chilonis* and *C. sesamiae*, are particularly difficult to distinguish. Prior studies investigated morphological and biological means of distinguishing members of this species complex. *Cotesia flavipes* can be distinguished from *C. sesamiae* and *C. chilonis* based on male genitalia (Polaszek and Walker, 1991), or through morphometric analysis (Sigwalt and Pointel, 1980; Kimani-Njogu *et al.,* 1997). Pupae of *C. sesamiae* and *C. flavipes* can be distinguished by spectroscopic methods (Cole *et al.*, 2003). Some species in the *C. flavipes* complex were distinguished using two to four genes (Smith and Kambhampati, 1999; Michel-Salzat and Whitfield, 2004), but relationships among all three members of the complex were not resolved. Differences in host finding, host suitability and mating compatability were identified for this species complex (Ngi-Song and Overholt, 1997; Potting *et al.*, 1997). *Chilo partelllus* and *Sesamia calamistis* (Hampson) are suitable for development of all three complex members (Okech and Overholt, 1996; Hailemichael *et al.*, 1997; Sallam *et al.*, 1999). *Busseola fusca* (Fuller) supported development of *C. sesamiae* from Kitale in western Kenya, but not a population of *C. sesamiae* from Mombasa on the eastern coast of Kenya, thus two strains of *C. sesamiae* exist (Mochiah *et al.*, 2001). Genetic differences of these two strains exist as well (Muirhead *et al.*, 2006).

Mating compatibility and courtship signals have been used to distinguish among parasitoid species difficult to distinguish based on morphology alone (Kimani and Overholt, 1997; Geden *et al.,* 1998; Pinto *et al.,* 2003). In mating crosses of the *C. flavipes* complex members, courtship signals appeared to differ among species (Kimani and Overholt, 1995). Male *C. sesamiae* did not wing fan in the presence of *C. flavipes* females, and female *C. sesamiae* did not mate with male *C. chilonis.* Acoustic courtship signals have discriminated among insect species and populations in orders including Plecoptera, Neuroptera, Heteroptera, Diptera, and Orthoptera (Claridge, 1985; Henry, 1994; Tomaru and Oguma, 1994; Stewart, 1997; Čokl and Virant-Doberlet, 2003; Rodriguez *et al.,* 2004; Cocroft and Rodriguez, 2005; Honda-Sumi, 2005), though rarely in Hymenoptera (van den Assem and Putters, 1980; Sivinski and Webb, 1989). Male parasitoid wasps fan their wings during courtship, producing low amplitude airborne sounds and substrate vibrations (Vinson, 1972; Kitano, 1975; Weseloh, 1977; Gordh and Debach, 1978; Vinson, 1978; van den Assem and Putters, 1980; Tagawa and Kitano, 1981; Wharton, 1984; Field and Keller, 1993a; Syvertson *et al.,* 1995; Ruther *et al.,* 2000). Courtship acoustics were specific for several species of Braconidae and Chalcidoidea (van den Assem and Putters, 1980; Sivinski and Webb, 1989; Rungrojwanich, 1994). Kimani and Overholt (1995) observed wing fanning behavior during courtship by males of the *C. flavipes* complex.

The first goal of this study was to compare substrate vibrations and airborne sounds produced by male *Cotesia* wing fanning during courtship using two recording methods, to determine if both methods recorded acoustic patterns of similar duration and frequency. Subsequently, the courtship acoustics were recorded and compared for two members of the complex, *C. flavipes* and *C. sesamiae*, with those of another noncomplex member, *Cotesia marginiventris* (Cresson). Finally, courtship acoustics were compared between two strains of *C. sesamiae*, one which develops in *B. fusca* and another which does not, in order to determine if differences occurred in courtship acoustics of the two strains.

Materials and Methods

Insect Rearing

A culture of *C. marginiventris* was maintained on larvae of the moth *Spodoptera frugiperda* (J. E. Smith). Parasitoid pupae were originally obtained from W. J. Lewis at U.S.D.A., A.R.S., Tifton Georgia. *Spodoptera frugiperda* adults were maintained in 2.4 L white plastic containers with 20% sugar: water solution as food, at 27°C ± 2°C, and 60% ± 5% relative humidity (RH). Adult moths oviposited on a paper towel, which was removed every 2 d, cut into strips (4 × 6 cm), and placed into 1 L glass mason jars with artificial wheat germ-based diet (Martinez *et al.,* 1988). Parasitoid adults were produced by placing one adult male and one female *C. marginiventris* (0-48 h old) in a 24 ml (6 dram) glass vial with ~ 20 *S. frugiperda* second instar larvae, larval diet, and a streak of honey for 48 h. *Spodopera frugiperda* larvae were then transferred in groups of 5 to plastic cups with diet until parasitoids formed cocoons. Each *C. marginiventris* pupa was isolated in a 1 ml (¼ dram) glass vial with a streak of honey for food and stoppered with cotton, so that emerging adults would remain virgin until used in an experiment. All adult *C. marginiventris* were stored overnight at 15 °C before using in mating trials, as this increased the likelihood of mating (A. J., unpubl. data).

The host for *C. flavipes*, *Diatraea saccharalis* (F.), was reared following methodology similar to that for *S. frugiperda*. The source of both *C. flavipes* and *D. saccharalis* was Weslaco, Texas, from a laboratory colony that was supplemented several times a year with field collected individuals. *Cotesia flavipes* was originally introduced into Texas from two sources, one from Pakistan and another from India (Fuchs *et al.*, 1979). Adult moths were held in 2.4 L plastic containers lined with wax paper for oviposition. Eggs on wax paper strips were placed in 1 L glass mason jars with diet. Single mated females of *C. flavipes* (0-48 h old) were placed in 10 ml plastic cups with two large *D. saccharalis* larvae (3^{rd}-6^{th} instar) and artificial diet, and incubated until parasitoid cocoons were visible. Individual *C. flavipes* cocoons were then placed singly in 1 ml (¼ dram) glass vials with a streak of honey so that emerging adults would remain virgin until used in an experiment.

The third species, *C. sesamiae*, was obtained as pupae from the International Center for Insect Physiology and Ecology (ICIPE), in Nairobi, Kenya. Two strains of *C. sesamiae* were obtained. One strain of *C. sesamiae* from Mombasa, on the east coast of Kenya, was reared at ICIPE on *S. calamistis;* this strain will not develop in *B. fusca*. The second strain was from Kitale, western Kenya, and reared on *B. fusca*. Two populations of the *B. fusca* strain were obtained, the one previously mentioned from Kitale, and another from Meru, central Kenya.

Recording Method Comparison: Laser and Microphone

All wasps used for recording were younger than 2 days old. Recordings were made at laboratory temperature, 25 ± 3º C, in an enclosed, sound reduction chamber (~1 m long × 1 m high × 0.75 m wide) on a vibration isolation table (TMC™, Model NAF 2000, Peabody, Massachusetts). Two species of parasitoids were used for comparing recording methods, the gregarious species *C. flavipes,* and the solitary species, *C. marginiventris.* One female and one male wasp of a species were placed in a plastic Petri dish (4 cm diam) with an organdy fabric bottom. A laser Doppler vibrometer (Polytec, Inc., Model OFV 353, Tustin, California) (1 mm/sec/volt sensitivity), was positioned above the Petri dish. The beam was focused on reflective tape ($4mm^2$) placed onto the organdy bottom of the Petri dish. A condenser microphone (AKG, Model C-1000, Nashville, Tennessee) with a frequency response of 20-20,000 Hz ± 2db, was positioned 0.5 cm below the arena, and both signals were digitized and recorded simultaneously on two channels using a Macintosh® computer equipped with an Audiomedia III sound card (16 bit, sampling rate 44.1 kHz). Peak software (version 3.0, Bias, Petaluma, California) was used to record the signals. Ten male-female pairs of each of the two species were recorded for 10 min, or less if mating occurred sooner. Recordings that had courtship sounds and vibrations detected were used for comparison.

Recording methods were assessed by comparing the airborne and substrate vibrations produced during courtship by *C. marginiventris* and *C. flavipes*. The pattern of courtship acoustics produced by both species has two parts (Fig. 2.1-2). The first part is a buzzing sound of longer duration and greater relative amplitude (hereafter ‘buzz’),

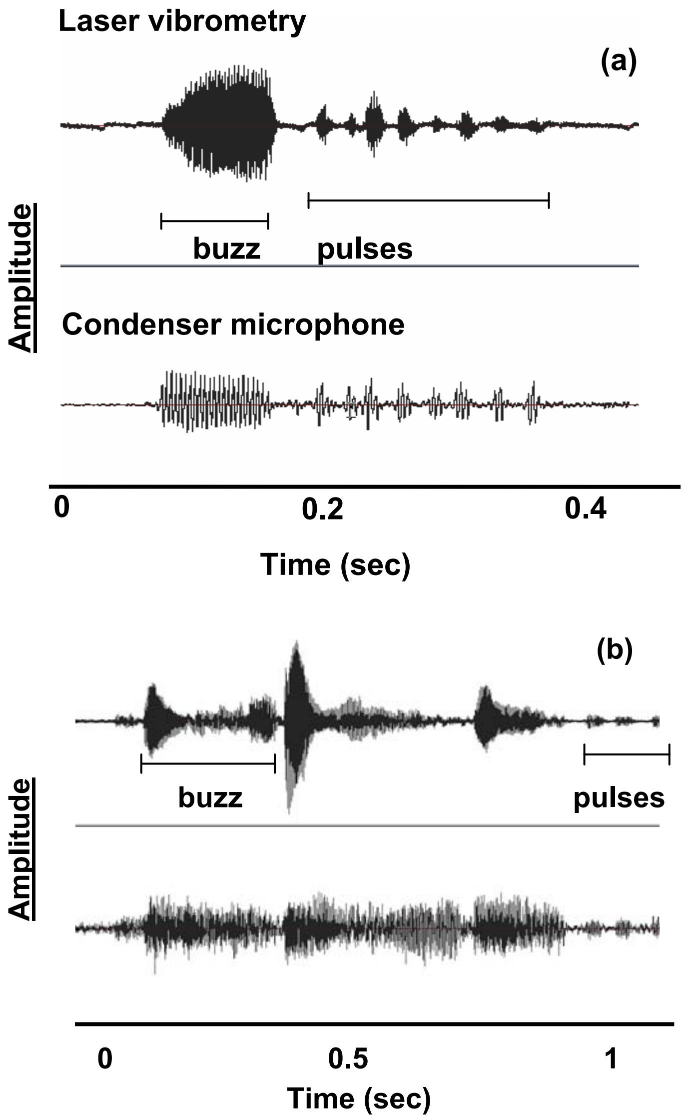

Fig. 2.1. Typical substrate and airborne vibration patterns produced by male wing fanning during courtship recorded with a laser Doppler vibrometer and a condenser microphone, respectively. (a) The *Cotesia flavipes* pattern has one long buzz preceded or followed by several short pulses, while (b) The *C. marginiventris* pattern has several long buzzes, which can be preceded or followed by a few short pulses.

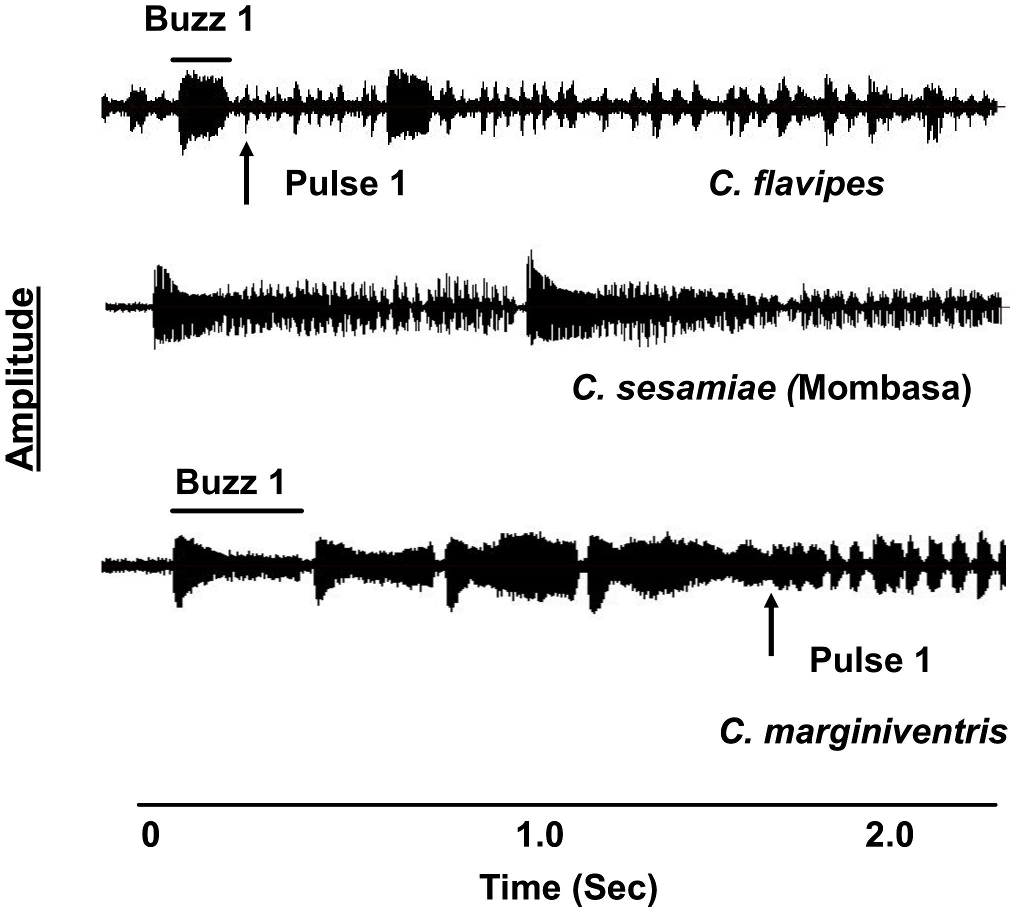

Fig. 2.2. Typical courtship acoustic patterns produced by male wing fanning in species of *Cotesia*, illustrating the buzz 1 and pulse 1 components.

followed by several pulses of relatively shorter duration and lower amplitude (hereafter 'pulse'). *Cotesia marginiventris* has several long buzzes, whereas *C. flavipes* includes only one long buzz (Fig. 2.2). A series of buzzes and pulses is hereafter referred to as a bout. The first buzz and pulse from the first bout produced by a courting male was used for analyses. Cool Edit Pro (Syntrillium software, now Audition, Adobe, San Jose, California) sound editing software was used to quantify the signals. The durations and fundamental frequencies of the buzz 1 and pulse 1 were measured (Fig. 2.2). For each signal parameter, data from the laser vibrometer and microphone were compared using paired *t*-tests. Statistical analyses were conducted using SPSS version 12.0 (SPSS, 2001).

Species Comparisons with a Condenser Microphone

The laser Doppler vibrometer and the condenser microphone recorded airborne and substrate vibration patterns with similar buzz 1 and pulse 1 durations as well as frequencies (see Results, Fig. 2.1). Subsequent recordings were thus made with the condenser microphone following the methodology described above. *Cotesia flavipes, C. sesamiae* (Mombasa strain), and *C. marginventris* were recorded for comparison among species. Recordings were conducted in the laboratory with overhead fluorescent lighting at 25 ºC ± 1 ºC.

Species comparisons were based on analysis of the first courtship bout produced by a male. The duration and frequency of the first buzz (buzz 1) and the first pulse (pulse 1) for each species were measured. Additional measurements included the interbuzz interval (the time interval between the start of buzz 1 and the start of the subsequent buzz 1) and interpulse interval (the time interval between the start of the first and second pulses). The interbuzz interval was only compared between *C. flavipes* and *C. sesamiae* (Mombasa), as the courtship acoustics pattern of *C. marginventris* contained more than one buzz in each bout.

Male courtship acoustics were recorded for 26 *C. marginiventris* males (13 that mated, and 13 that did not), 24 *C. flavipes* males (14 mated and 10 unmated), and 14 *C. sesamiae* (Mombasa) males (7 mated and 7 unmated). Variances were not homogeneous for the parameters buzz 1 duration, pulse 1 duration, interbuzz interval, and interpulse interval (SPSS, Levene's test). Log transformation was sufficient to normalize the variance for buzz 1 duration, so corresponding data were analyzed by two-way ANOVA, with species and mating status as main effects, followed by a Tukey's test to separate means (Sokal, 1995). Pulse 1 duration, interbuzz interval, and interpulse interval data were subjected to non-parametric procedures. The Scheirer-Ray-Hare test was used to compare pulse 1 durations and interpulse intervals among the three species, followed by a Games Howell post-hoc test for means with unequal variances (Sokal, 1995). A Mann-Whitney U test was used to compare the interbuzz interval between *C. flavipes* and *C. sesamiae* (Mombasa) (Siegel and Castellan, 1988). Buzz 1 frequency and pulse 1 frequency were analyzed using two-way ANOVA, with species and mating status as main effects; means were separated as warranted by Tukey's tests.

Comparing Populations of **C. sesamiae** *with* **C. flavipes**

Courtship acoustics of two additional populations of *C. sesamiae* were recorded. One population of *C. sesamiae* was from Kitale, western Kenya (hereafter Kitale), and the second population was from Meru (hereafter Meru), central Kenya. Both *C. sesamiae* (Kitale) and *C. sesamiae* (Meru) were reared at ICIPE on *B. fusca* and are considered the same strain, as both develop on *B. fusca* and *S. calamistis.* These two populations differ biologically from *C. sesamiae* (Mombasa) which does not develop on *B. fusca*. The courtship acoustics of *C. sesamiae* (Kitale) and *C. sesamiae* (Meru) were recorded in the laboratory with a condenser microphone, with fluorescent lighting at 25 ºC ± 1 ºC, using the methodology described above. There were 20 male-female pairs from the *C. sesamiae* (Kitale) and 17 from the *C. sesamiae* (Meru) populations.

Data were collected from the first courtship bout produced by a courting male. The duration and frequency of buzz 1 and pulse 1 was measured for each population, as was the interbuzz interval and interpulse interval. Data from *C. sesamiae* (Kitale) and *C. sesamiae* (Meru) were compared to *C. sesamiae* (Mombasa) and *C. flavipes*. Buzz 1 frequency and pulse 1 frequency data were analyzed using ANOVA. Variances were not homogeneous for the parameters buzz 1 duration, pulse 1 duration, interbuzz interval and interpulse interval (SPSS, Levene's test). Log transformation normalized the variance for buzz 1 duration, so ANOVA was conducted on log transformed data, followed by a Tukey's test (Sokal, 1995; SPSS, 2001). A Kruskall-Wallis test was used to analyze data for pulse 1 duration, interbuzz interval and interpulse interval, followed by a post-hoc separation of means (Siegel and Castellan, 1988). Voucher specimens for all insect species and populations are deposited in the Texas A&M Department of Entomology Insect Collection, voucher number 668.

Results

Recording Method Comparison: Laser and Microphone

Differences between mean durations and frequencies of courtship acoustic patterns recorded with a laser Doppler vibrometer and a condenser microphone were not significant for either *C. marginiventris* or *C. flavipes* (Table 2.1, Fig. 2.1). The fundamental frequencies of both species courtship acoustics were between 260-314 Hz (Table 2.1).

Species Comparison

Courting male *C. marginiventris* typically produced sounds and vibrations that consisted of three long buzzes, preceded or followed by a series of shorter pulses, while those of *C. flavipes* and *C. sesamiae* (Mombasa) had one long buzz and numerous short pulses (Fig. 2.2). However, buzz 1 for *C. flavipes* was shorter in duration and ended more abruptly than the buzz 1 for *C. sesamiae* (Mombasa) (Fig. 2.2). The duration of buzz 1 differed among species (ANOVA, $F_{2,62} = 279.76$, $P < 0.001$), but was not affected by mating status ($F_{1,62} = 0.12$, $P = 0.74$), and there was no interaction between species and mating status ($F_{2,62} = 0.87$, $P = 0.43$). *Cotesia flavipes* had a shorter buzz 1 duration than *C. sesamiae* (Mombasa) or *C. marginiventris* (Fig. 2.3a). .

Table 2.1. Comparison of courtship acoustic parameters obtained using a laser Doppler vibrometer and a condenser microphone, from *C. marginiventris* and *C. flavipes*, using a paired *t*-test for each call parameter.

Species	Call Parameter	Laser Vibrometer (Mean ± S. E.)	Microphone (Mean ± S. E.)	n	*P*	*t*
C. marginiventris	Buzz 1 duration (ms)	279.33 ± 16.68	279.17 ± 16.67	6	0.36	1.00
	Buzz 1 frequency (Hz)	304.03 ± 9.42	303.15 ± 9.71	6	0.36	1.0
	Pulse 1duration (ms)	37.00 ± 3.94	36.75 ± 3.86	4	0.39	1.0
	Pulse 1 frequency (Hz)	261.60 ± 12.56	261.50 ± 12.55	4	0.39	1.0
C. flavipes	Buzz 1 duration (ms)	85.25 ± 3.69	84.25 ± 3.65	8	0.09	2.0
	Buzz 1 frequency (Hz)	284.63 ± 10.42	280.50 ± 10.23	8	0.11	1.9
	Pulse 1 duration (ms)	12.63 ± 1.87	12.50 ± 1.92	8	0.35	1.0
	Pulse 1 frequency (Hz)	267.00 ± 14.49	266.86±14.57	7	0.36	1.0

Similarly, the buzz 1 frequency was influenced by species (ANOVA, $F_{2,63} = 67.87$, $P < 0.001$), but not by mating status ($F_{1,63} = 0.14$, $P = 0.72$), and there was no interaction ($F_{2,63} = 2.29$, $P = 0.11$). All pairwise comparisons by Tukey's test were significant (Fig. 2.3b). The buzz 1 frequency for *C. flavipes* was highest, for *C. marginiventris* was intermediate, and was lowest for *C. sesamiae* (Mombasa) (Fig. 2.3b). The duration of pulse 1 differed significantly among the three species (Scheire-Ray-Hare*,* $H_2 = 34.56$, $P < 0.001$), but was not affected by mating status ($H_1 = 0.41$, $P = 0.52$), and there was no significant interaction between species and mating status ($H_2 = 1.03$, $P = 0.6$). Pulse 1 durations of *C. flavipes* and *C. sesamiae* (Mombasa) were not significantly different, both were shorter than *C. marginiventris* (Fig. 2.3c). The pulse 1 frequency differed among species (ANOVA, $F_{2,57} = 47.97$, $P < 0.001$), but was not influenced by mating status ($F_{1,57} = 0.89$, $P = 0.35$), and there was no interaction between species and mating status ($F_{2,57} = 0.58$, $P = 0.5$). *Cotesia flavipes* had the highest pulse 1 frequency, *C. marginiventris* was intermediate, and *C. sesamiae* (Mombasa) was lowest (Fig. 2.3d). The buzz 1 interbuzz interval was significantly different between *C. flavipes* and *C. sesamiae* (Mombasa) (Mann-Whitney U, $Z = 2.10$, N1 = 22, N2 = 13, $P = 0.04$). Finally, the interpulse interval differed among the three species (Scheire-Ray-Hare, $H_2 = 34.14$, $P < 0.001$), but was not influenced by mating status (Scheire-Ray-Hare, $H_1 = 0.02$, $P = 0.90$), and there was no significant interaction (Scheire-Ray-Hare, $H_2 = 1.20$, $P = 0.55$). The mean interpulse durations of all three species were significantly different (Games Howell, $P < 0.50$).

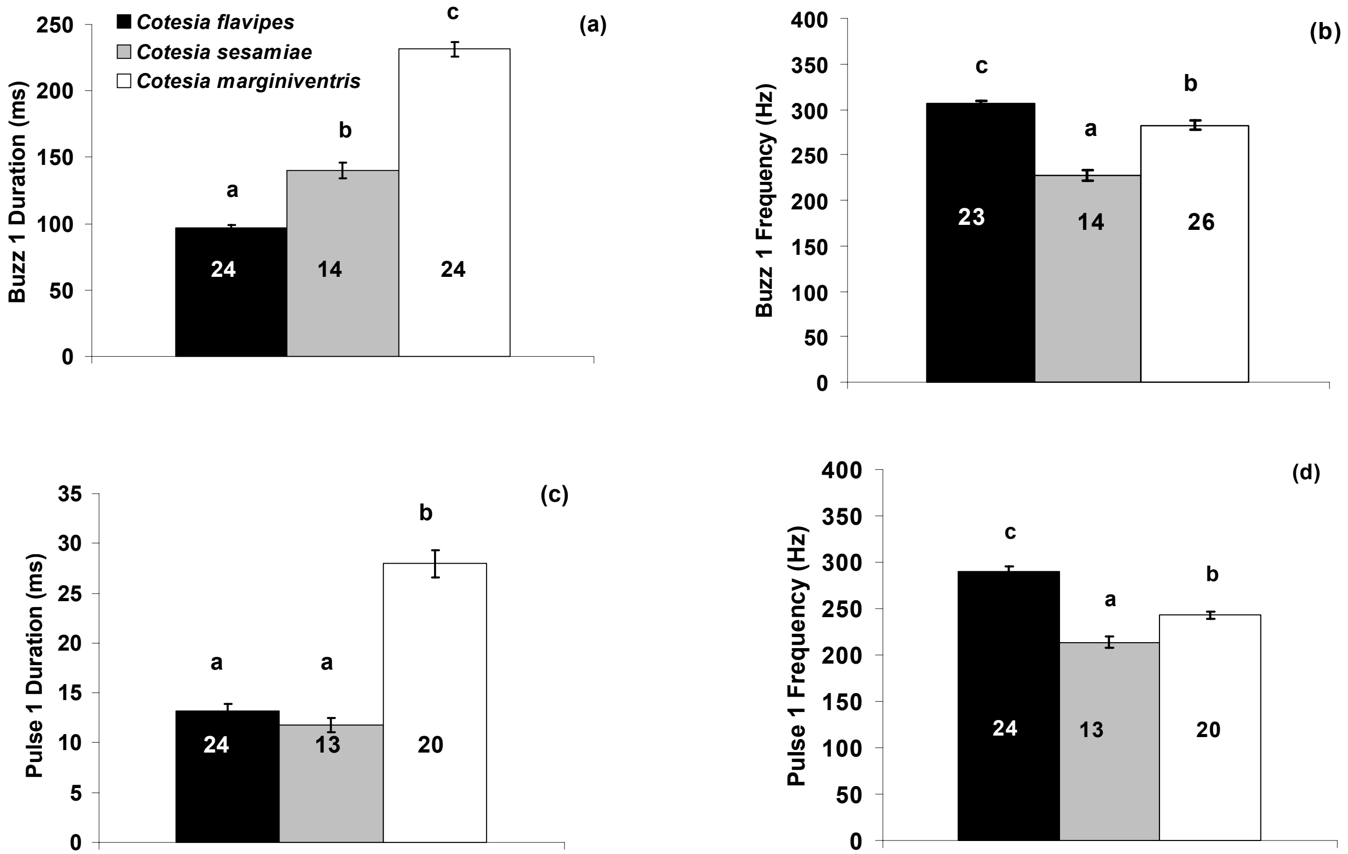

Fig. 2.3. Mean (± S.E.) courtship acoustic parameters of three *Cotesia* species. Species had a significant influence on (a) Buzz 1 duration (ANOVA, $P < 0.001$), (b) Buzz 1 frequency (ANOVA, $P < 0.001$), (c) Pulse 1 duration (Scheire-Ray-Hare, $H_2 = 34.56$, $P < 0.001$), means separation by Games Howell test ($P < 0.05$), and (d) Pulse 1 frequency (ANOVA, $P < 0.001$). Different lower-case letters above columns indicate significant differences (Tukey's test, $P < 0.05$).

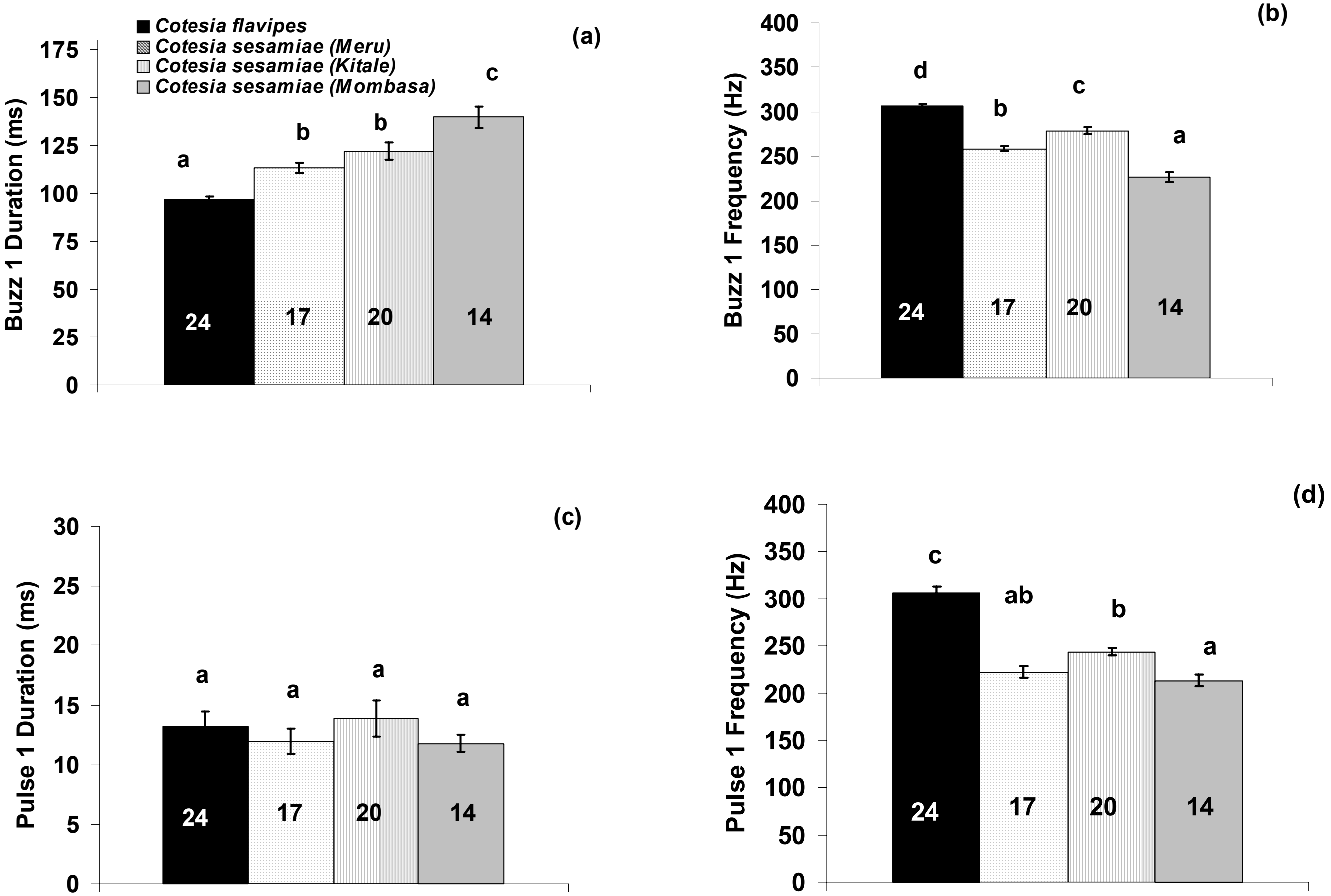

Fig. 2.4. Mean (± S.E.) courtship acoustic parameters of four *Cotesia* populations. (a) Buzz 1 duration differed among populations (ANOVA, $P < 0.001$). (b) Populations significantly influenced buzz 1 frequency (ANOVA, $P < 0.001$). (c) Pulse 1 duration was not significantly different among populations (Kruskall-Wallis, $P < 0.93$) (d) Pulse 1 frequency differed among populations (ANOVA, $P < 0.001$). Different letters above columns indicate significant differences (Tukey's, $P < 0.05$).

Comparing Populations of **C. sesamiae** *with* **C. flavipes**

Cotesia sesamiae (Kitale) and *C. sesamiae* (Meru), two populations of a strain of *C. sesamiae* that develop in *B. fusca,* both had one long buzz and numerous pulses in their courtship acoustic pattern, as seen for *C. sesamiae* (Mombasa) and *C. flavipes* (Fig. 2.2). The buzz 1 duration differed among the four populations (ANOVA, $F_{3,71} = 24.54$, $P < 0.001$), and was shorter for *C. flavipes* than the populations of *C. sesamiae* (Fig. 2.4a). However, *C. sesamiae* (Mombasa) had a longer buzz 1 duration than the other strain from *C. sesamiae* (Kitale) or *C. sesamiae* (Meru) (Fig. 2.4a). Similarly, the buzz 1 frequency was significantly different among all populations (ANOVA, $F_{3,70} = 68.05$, $P < 0.001$) (Fig. 2.4b), and higher for *C. flavipes* than for the populations of *C. sesamiae*. Pulse 1 duration did not differ among the four populations (Kruskal-Wallis, $\chi_2 = 0.45$, $P < 0.93$) (Fig. 2.4c). The pulse 1 frequency differed among populations (ANOVA, $F_{3,70} = 35.78$, $P < 0.001$), with *C. flavipes* higher than the three *C. sesamiae* populations, and no significant difference among the three populations of *C. sesamiae* (Fig. 2.4d). The interbuzz interval was not significantly different among these four populations (Kruskall-Wallis, $\chi^2 = 4.44$, P <0.22). Interpulse intervals (Kruskal-Wallis, $\chi^2 = 12.99$, $P < 0.005$) were significantly different among the four populations, with the interpulse interval for *C. flavipes* longer than for *C. sesamiae* (Kitale) and *C. sesamiae* (Mombasa), there was no significant difference in interpulse interval among the *C. sesamiae* populations.

Discussion

Wing fanning by courting males of *C. flavipes* and *C. marginiventris* produced both substrate vibration and airborne sound patterns that were similar in duration and frequency as measured by the laser vibrometer and the condenser microphone (Fig. 2.1a, 2.1b). The substrate vibrations produced during courtship may be from movement of the wing muscles, as in species of *Drosophila* Fallén (Tauber and Eberl, 2003). In a study of the parasitoid *Nasonia vitripennis* (Walker), courtship acoustics from male wing fanning were recorded with both a phonograph cartridge (to record substrate vibration) and a microphone, and resulting recordings by both methods were comparable (van den Assem and Putters, 1980), as in the present study. Other insects produce similar airborne and substrate vibrational patterns during courtship (Heady *et al.*, 1986; Stotling *et al.*, 2002). However, some insects produce patterns of airborne courtship sounds that differ from the courtship substrate vibrations (DeLuca and Morris, 1998), while some insects produce substrate vibrations only (Henry *et al*., 2002).

In this study, the courtship acoustic pattern for *C. marginiventris* was similar to that reported for this species by Sivinski and Webb (1989), consisting of 1-8 long buzzes, preceded or followed by short pulses (Fig. 2.2). Also, the range of buzz frequencies for *C. marginiventris* found in this study (280-304 Hz) was similar to the frequency of 314 Hz reported by Sivinski and Webb (1989). The two populations are from different locations, one from Tifton, Georgia, the other from Gainesville, Florida, though the geographic limits of the source populations are unknown. The courtship acoustics of the *C. flavipes* complex had not been previously recorded, though wing fanning during courtship had been observed (Kimani and Overholt, 1995). Parasitoids are known to have species-specific courtship acoustics, but different populations from a species have not previously been compared (van den Assem and Putters, 1980; Sivinski and Webb, 1989; Rungrojwanich, 1994). Typical recordings of *C. flavipes* and three populations of *C. sesamiae* exhibited one long buzz and numerous pulses, a pattern which differed in structure from the 1-8 long buzzes produced by courting *C. marginiventris* males. The buzz 1 durations differed between *C. flavipes* and *C. sesamiae* (Mombasa) (Figs. 2.2, 2.3a). In addition, the buzz 1 frequency, pulse 1 frequency, interbuzz interval, and interpulse interval were all distinct for each of the three *Cotesia* species. *Cotesia flavipes* and *C. sesamiae* are allopatrically distributed, so may not be under selective pressure to differentiate courtship signals through character displacement, which might result if they were sympatric (Butlin, 1995; Gerhardt and Huber, 2002; Hobel and Gerhardt, 2003).

Two other parasitoid wasps in the family Braconidae, *Diachasmimorpha longicaudata* (Ashmead) and *D. kraussii* Fullaway, that are allopatrically distributed have courtship acoustics with similar patterns, but they have significantly different pulse durations and pulse intervals (Rungrojwanich, 1994; Rungrojwanich and Walter, 2000). Heady *et al.* (1986) found that 8 of 10 species of *Dalbulus* DeLong had distinct courtship songs, but three allopatric species were not significantly different. Differences in courtship sounds of allopatric species, such as those of the *C. flavipes* complex, could be due to factors such as genetic drift (Čokl *et al*., 2000). The courtship acoustics of sympatric species may differentiate more than allopatric ones in order to prevent hybridization (Gerhardt and Huber, 2002). Species with sympatric distributions have divergent courtship songs, such as *Drosophila pseudoobscura* Frolova and *D. persimilis* Dobzansky and Epling, which varied significantly in the interpulse interval and intrapulse frequency (Noor and Aquadro, 1998). Two sympatrically distributed cricket species, *Gryllus texensis* Cade and Otte and *G. rubens* Scudder, had significantly different acoustic calls, as did the two stinkbugs *Thyanta pallidovirens* (Stål) and *T. custator accerra* McAtee, which had distinct vibrational courtship signals (Fitzpatrick and Gray, 2001; McBrien *et al.*, 2002).

Significant differences were evident in the courtship acoustics among populations of *C. sesamiae*. The buzz 1 duration differed between *C. sesamiae* (Mombasa) and *C. sesamiae* (Kitale) or (Meru), but did not vary within the two populations of *C. sesamiae* (Kitale, Meru) of the strain that develops in *B. fusca*. Buzz 1 frequency was different for all four populations, pulse 1 duration did not differ among populations, and pulse 1 frequency of *C. flavipes* was higher than all populations of *C. sesamiae* (Figs. 2.3b-d). Differences in courtship behavior might be expected between the two *C. sesamiae* strains, as they are reported to have significant genetic differences (Muirhead *et al.*, 2006). Biological differences in host suitability exist for the two strains as well (Mochia *et al.,* 2001). The populations studied here were all allopatric populations, yet displayed significant variation in the courtship acoustic patterns.

Allopatric populations of other insects have shown significant differences in courtship acoustics, including populations that are not reproductively isolated. Two allopatric populations of *Teleogryllus oceanicus* (le Guillou) crickets had distinct courtship songs that varied at the extremes of their geographic ranges, in Hawaii and Australia, and females preferred songs with longer chirps (Simmons, 2004). Two populations of *Nezara viridula* (L.), from Slovenia and Australia, had courtship songs with different temporal patterns, and a low mating rate (3%) between the populations (Ryan *et al.*, 1996). Čokl *et al.* (2000) examined four populations of *N. viridula*, from Brazil, Florida, Italy, and Slovenia. The populations from Italy and Florida had courtship song pulse durations more similar to each other than to the populations of *N. viridula* from Brazil and Slovenia, and they intermated.. Recordings of the green lacewing *Chrysoperla carnea* (Stephens) found at least three song types, which may be indicative of distinct species (Henry *et al.,* 2002). The courtship acoustics of the *C. flavipes* complex, along with morphology, host suitability, and molecular characteristics, can be used for separating or distinguishing populations or species in this complex. Applying the methodology presented in this study to other populations of *C. sesamiae* in Kenya may reveal the presence of additional strains with differential host preferences.

Courtship behavior in the *Cotesia* species that were investigated could be mediated by near-field sound or substrate vibrations. Hymenoptera are known to use either mode of communication (Towne and Kirchner, 1989; Wackers *et al.,* 1998; Meyhöfer and Casas, 1999; Cocroft, 2001; Greenfield, 2002). The mean fundamental frequency of the courtship acoustics of all three *Cotesia* species was approximately 300 Hz, within the range produced or detected by other hymenopteran parasitoids (van den Assem and Putters, 1980; Sugimoto *et al.,* 1988; Sivinski and Webb, 1989). *Cotesia* courtship acoustics may play a role in species recognition or mate choice (Chapter IV).

Courtship signals in many insects and vertebrates consist of multiple components, which may function in species recognition or mate choice (Gerhardt and Huber, 2002). Other insects have calling or courtship signals consisting of several components (Hunt and Morton, 2001; Tauber and Eberl, 2003). *Graminella nigrifrons* (Forbes) (Cicadellidae) has a vibrational calling song consisting of three components, and females respond primarily to the second and third components (Hunt *et al.*, 1992). *Drosophila* species have two-part courtship signals, including the pulse and sine songs, of which the interpulse interval is considered species specific (Tauber and Eberl, 2003). This study showed that the courtship acoustics of all three *Cotesia* species have at least two parts, the buzz and pulse components. The courtship acoustics of *C. marginiventris* appeared more complex with numerous long buzzes, relative to those of *C. flavipes* or *C. sesamiae*, which had only one long buzz (Fig. 2.2). *Cotesia marginventris* is a solitary species (Tillman, 2001) that presumably disperses to locate mates, and may encounter heterospecifics, as well as greater habitat variation due to the broad host plant range of some of its hosts. The more complex signal of *C. marginiventris* may enhance species recognition in the presence of heterospecifics, and in variable environments, or could be a result of sexual selection. In contrast, *C. flavipes* and *C. sesamiae* are gregarious (Tagawa and Kitano, 1981; Arakaki and Ganaha, 1986), and are likely to exhibit significant levels of sibmating, as seen in another gregarious species, *C. glomerata* (Gu and Dorn, 2003). *Cotesia flavipes* attacks stemborer larvae that are pests of grasses, and thus encounters a less diverse host plant range than *C. marginiventris*. Courtship acoustics may be more important for *C. marginiventris* than for *C. flavipes*.

In summary, the present study showed that courtship acoustics can be used to help differentiate closely related species of parasitoids or strains in the genus *Cotesia*. Further study of the role of courtship acoustics in species recognition and mate preference could provide important insights into their function, and could be used in conjunction with other methodologies to investigate whether additional strains or species exist in the *C. flavipes* complex.

CHAPTER III

THE ROLE OF SUBSTRATE ON MATING, COURTSHIP VIBRATION TRANSMISSION, AND ACOUSTIC COMMUNICATION IN *Cotesia marginiventris*

Introduction

Insect mating is influenced by both chemical and physical environmental factors, such as host plant odors, ambient temperatures, and time of day (van den Assem, 1986; Quicke, 1997), but the influence of the substrate and its physical properties on mating have rarely been investigated. When investigated, the mating success of some insects and arthropods that use vibrational communication was shown to be affected by the mating substrate and its physical properties. For example, males of the jumping spider, *Habronattus dossenus* Griswold (Araneae: Salticidae), courted females on several natural substrates including rock, sand, and leaf litter, but mating frequency was greatest on leaf litter (Elias *et al.*, 2004). Males of *Nezara viridula* (L.) (Pentatomidae) responded more strongly to courtship vibrations on a plant than on a loudspeaker (Miklas *et al.*, 2001) Courtship vibrations of *Umbonia crassicornis* Amyot and Serville treehoppers transmitted in a similar manner through two natural substrates, a host and non-host woody plant (Cocroft *et al.*, 2006), as did those produced by *Chrysoperla downesi* (Smith) and *C. plorabunda* (Fitch) lacewings courting on grass and hemlock (Henry and Martinez Wells, 2004).

Male parasitoid wasps from several families of Hymenoptera, including males of *Cotesia* in the family Braconidae, fan their wings during courtship, producing low amplitude sounds and substrate vibrations, which may be used for communication with females (Leonard and Ringo, 1978; van den Assem and Putters, 1980; Sivinski and Webb, 1989; Field and Keller, 1993a). Courtship vibrations appear to be critical for mating in *Cotesia rubecula* (Marshall) (Field and Keller, 1993a), and courtship acoustics of *Nasonia vitripennis* (Walker) (Pteromalidae) may indicate male quality (van den Assem and Putters, 1980). The relevance, if any, of these courtship vibrations in relation to insect mass rearing programs has not been addressed, yet ensuring that insects mate is essential for efficient rearing. Courtship vibrations may transmit differentially in materials commonly used in mass rearing programs, such as plastic, glass, fabric, and plants, as they have been shown to transmit differentially in materials used for building and sound proofing (Rossing and Fletcher, 2004). Rearing substrates may affect the vibrational communication between courting parasitoids, which could affect the mating rate among females. Mated female parasitoids produce female and male offspring, while virgin females produce only males. A common goal in parasitoid mass rearing is to produce as many females as possible, because only females attack and kill hosts in the field (Heimpel and Lundgren, 2000). Thus, greater efficiency in parasitoid mass rearing could be obtained by increasing the frequency of mated females in a colony. Rearing parasitoid wasps that are known or suspected to rely on vibrational courtship signals may be improved by appropriately selecting substrates that will facilitate mating.

Cotesia marginiventris (Cresson) (Hymenoptera: Braconidae) is a parasitoid wasp whose hosts are moth larvae (Noctuidae), and is considered an important natural control agent of several pest species, including *Spodoptera fruigiperda* (J. E. Smith) and

Trichoplusia ni (Hüber) (McCutcheon and Turnipseed, 1981; Braman and Yeargan, 1991; Novoa and Luna, 1996; Gillespie *et al*., 1997). *Cotesia marginiventris* is a solitary endoparasitoid that attacks first and second instar larvae (Boling and Pitre, 1970; Kunnalaca and Mueller, 1979; Tillman, 2001), and has been considered for augmentative biological control of *T. ni* in greenhouses (Gillespie *et al.,* 1997; Urbaneja *et al.,* 2002; Riddick, 2006). However, *C. marginiventris* sex ratios recorded under mass rearing and field conditions vary between 20 and 60% females (Jalali *et al*., 1987; Riggen *et al.,* 1992; Novoa and Luna, 1996; Gillespie *et al*., 1997; Tillman, 2001). The low sex ratios observed in some studies may be due to a high frequency of virgin females, though the potential role of mating substrates and transmission of courtship vibrations in rearing materials were not investigated in those studies.

The objective of this study was to assess the influences of natural and artificial rearing substrates on the mating success and transmission of courtship vibrations for the parasitoid *C. marginiventris*. In addition, the importance of airborne relative to substrate vibrations produced during courtship was assessed by experimentally manipulating the courtship vibrations and assessing their influence on the mating success of *C. marginiventris* females.

Materials and Methods

Insects

Cotesia marginiventris wasps were reared on *Spodoptera frugiperda* as previously described (Chapter II). Prior to all trials, individual parasitoid cocoons were isolated in glass shell vials (1 ml, 30 × 8 mm, Bioquip, Gardena, California) to prevent adults from mating. Each experimental arena and all parasitoids were used only once and then discarded.

Mating Success on Five Substrates

The mating success of individual *C. marginiventris* females was determined on five substrates associated with parasitoid rearing, glass shell vials (1ml, 30 × 8 mm, Bioquip, Gardena, California), plastic Petri dishes (50 × 9 mm, Falcon®, Becton Dickinson Labware, Franklin Lakes, NJ), white chiffon fabric (Hancock Fabrics, Chiffon Georgette, 100% polyester), and two host plant substrates, corn leaves (*Zea mays* L., Pioneer® seed (34A55) (Johnston, Iowa, U.S.A.), and bean leaves (*Vigna unguiculata* (L.), California blackeye cowpea #5, Gurney's Seed and Nursery Co., Greendale, Indiana). The chiffon fabric arena consisted of a plastic Petri dish (50 × 9 mm) with a circular opening (40 mm diam) cut in the lid, covered by chiffon fabric. The corn and bean leaf arenas consisted of a leaf, which remained attached to the plant, covered with a plastic vial (160 ml, 85 × 50 mm, Bioquip, Gardena, California) to confine the parasitoids. Glass shell vials were closed with a small piece of cotton, to prevent parasitoids from escaping.

Male-female pairs of virgin parasitoids were monitored for 10 min on each of the five substrates at laboratory temperature and humidity (25 ± 2 °C, 50% rh), between 08:00 and 10:00 h, and mating success or failure was recorded. All parasitoids were 2-4 d old, and 34 pairs were observed per substrate. A Chi-square goodness of fit test was used to determine whether mating frequencies differed among the five substrates. Post hoc separation of mating frequencies on each substrate was by a Tukey-type test for proportions (Zar, 1999).

Transmission of Courtship Vibrations on Five Substrates

Courtship vibrations produced by male *C. marginiventris* were recorded on the five substrates described above, glass vials, plastic Petri dishes, chiffon fabric, and bean and corn leaves, in order to determine the influence of the substrate on the transmission of courtship vibrations. All parasitoids were virgin, 2-4 d old, and used for only one recording. Males were recorded courting a female on each substrate for 10 min. Recordings were made using a randomized block design. Each block consisted of a single replicate (male-female pair) on each of the five substrates, and the order of substrates was randomized within each block. Ten pairs were recorded on each substrate.

Recordings were made with a Doppler laser vibrometer (Polytec, Inc., Model OFV 353, Tustin, California) at 1 mm/sec/volt sensitivity at laboratory temperature, 25 ±

3º C, in an enclosed, sound reduction chamber (~1m long × 1m high × 0.75m wide), on a vibration isolation table (TMC™, Model NAF 2000, Peabody, Massachusetts). One female-male pair was placed on one of the substrates. The laser was positioned above the arena, and the laser beam was focused on reflective tape ($4mm^2$) placed on the surface of the test substrate. The vibrational signal detected by the laser was digitized and recorded on a Macintosh® computer equiped with an Audiomedia III sound card (16 bit, sampling rate 44.1 kHz). Peak® software (version 3.0, Bias, Petaluma, California) was used to record the signals. The distance between the laser beam point of contact on the substrate and the location of the courting male was monitored and recorded in 1 cm increments. Only courtship sounds produced by males within 2 cm of the laser point of contact on the substrate were used for analyses.

Vibrations produced by courting *C. marginiventris* males consist of several long buzzes, which may be preceded and/or followed by shorter pulses (Chapter II). Several parameters of the buzz 1 component produced by courting males were used for comparison of vibration transmission among the five substrates (Fig. 3.1). Studies of insect acoustics often characterize signals by measuring the duration, frequency and amplitude (Ewing, 1989). The buzz 1 duration was measured in ms. The fundamental frequency (Hz) and its relative amplitude (db) were measured at both the beginning and end of buzz 1, i.e. 10 ms into the start of buzz 1, and 10 ms before the end of buzz 1, to determine the amount of frequency modulation (change) in the courtship vibrations between the beginning and the end of the signal, and whether a substrate filtered out some frequencies and not others. The frequency and relative amplitude were also measured for the second and third harmonics of buzz 1. Relative amplitudes, rather than absolute amplitudes, were measured because it was not practical to calibrate each measurement while maintaining parasitoid age constant for recordings. In addition, a ratio of the relative amplitude of the fundamental harmonic to that of the second and third harmonics (i.e. fundamental harmonic/second harmonic; fundamental harmonic/third harmonic) was calculated for the beginning and end of buzz 1, to determine the relative amplitude of the fundamental harmonic to the second and third harmonics in each substrate. Background noise was considered constant in all recordings, as all recordings were under standardized laboratory conditions, using a randomized design.

Statistical comparison of each courtship parameter among the substrates was by one-way analysis of variance, with Tukey's post-hoc test, for variables that met assumptions of ANOVA (SPSS, 2001); nonparametric Kruskal-Wallis tests were used to compare the buzz 1 duration, fundamental frequency at the end of buzz 1, frequency at the end of the second harmonic, and mean ratios of relative amplitudes among the five substrates (Statistix, 2000), with posthoc pairwise comparisons as described in Siegel and Castellan (1988). Finally, within a substrate, both the frequency and the amplitude were compared between the beginning and end of buzz 1, to determine if a significant change occurred in that signal parameter, using a paired *t*-test. Wilcoxon Signed-Rank tests were used when assumptions of *t*-tests were not met (SPSS). All *t*-tests were two tailed.

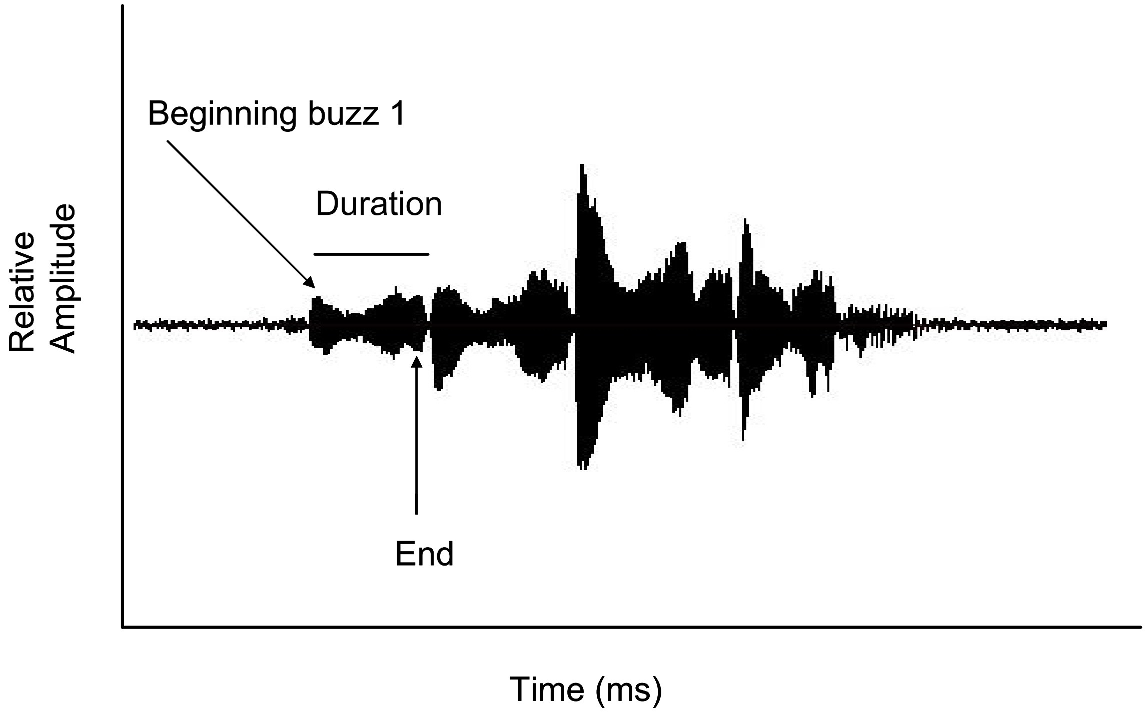

Fig. 3.1. A typical bout of substrate vibrations produced by courting *C. marginiventris* males, showing the buzz 1 component used for statistical comparisons among mating substrates.

Role of Airborne and Substrate Vibration in Mating

Two experiments were conducted to assess the importance to mating success of airborne relative to substrate vibrations produced by courting males. The first experiment compared the mating success of normal and dealated males (wings ablated at the base) on a good (chiffon) and poor (glass) mating substrate, according to the mating success and transmission characteristics shown for those substrates in prior experiments (see Results, *Mating Success on Five Substrates*, and *Transmission of Courtship Vibrations on Five Substrates*). If courtship signaling relied exclusively on near-field sound (airborne), then wing ablation, but not substrate, should affect mating success. In contrast, if courtship signaling relied exclusively on substrate vibration, then substrate and not wing ablation, should affect the mating success of males.

Both dealated and normal males were prepared for trials by placing in vials and chilling in a freezer for 10 min and then removing and placing the vials on a frozen cold pack. Each male was then placed dorsal side up under a dissecting microscope, and the thorax was pressed gently with forceps to spread the wings. Dealated males were prepared by ablating each of the wings near the base, leaving approximately ¼ of each wing. Normal males were mock ablated, using the forceps to press on the thorax and touch the wings, but wings were otherwise left intact.

Each male, normal or dealated, was paired individually with a female for up to 10 min and mating success or failure was recorded. Eighteen normal and 18 dealated males were tested on both chiffon and glass. Statistical analysis was by logistic regression, with mating as the dependent variable (mated or not mated), and two independent predictor variables, substrate (glass or chiffon) and wings (normal or dealated), to assess the relative importance of wings and substrate (Stata, 2005).

The second experiment was to asses whether wing ablation affected substrate vibration produced by courting males. This was done by comparing the amplitude of substrate vibrations produced by courting normal and dealated males on the chiffon arena. A calibration procedure for amplitude of substrate vibration was performed prior to each recording in order to measure amplitude of substrate vibration in velocity units (mm/s). Velocity is the speed (loudness/amplitude) the substrate is moving due to vibration. A courting male and a female in an chiffon arena were recorded with a laser vibrometer as described above, and data were captured using SpectraPro® software (Sound Technology, Aylesbury, UK).

Courting dealated males were recorded prior to and after wing ablation, as were courting normal males prior to and after mock wing ablation, to determine the magnitude of any change in courtship vibration amplitude. The first courting bout for each male was used to measure peak velocity of the courtship vibration amplitude. Paired *t*-tests were used to compare the peak velocities for dealated males prior to and after ablation, and for normal males prior to and after mock ablation.

Results

Mating Success on Five Substrates

The mating success of females differed among substrates (χ_4^2= 17.74, $P = 0.001$). Mating success was greatest on chiffon, intermediate on corn and bean, and lowest on glass and plastic (Fig. 3.2).

Transmission of Courtship Vibration on Five Substrates

The duration of the buzz 1 varied significantly among substrates (Kruskal-Wallis, χ_4^2 23.22, $P < 0.001$). The duration on plastic was significantly shorter than on corn, bean and chiffon, and the duration on glass was intermediate between plastic and the other substrates (Fig. 3.3).

The fundamental frequency at the beginning of buzz 1 differed among the 5 substrates (ANOVA, $F_{4, 45} = 16.24$, $P < 0.04$). Bean had a lower mean frequency than glass, while the fundamental frequencies on other substrates were not significantly different (Fig. 3.4a). Fundamental frequencies among the five substrates at the beginning of buzz 1 were 300-325 Hz (Fig. 3.4a). At the end of buzz 1 fundamental frequencies differed significantly among substrates (Kruskal-Wallis, χ_4^2 31.22, $P < 0.001$), with corn, bean, and chiffon having lower mean frequencies than plastic or glass (Fig. 3.4a).

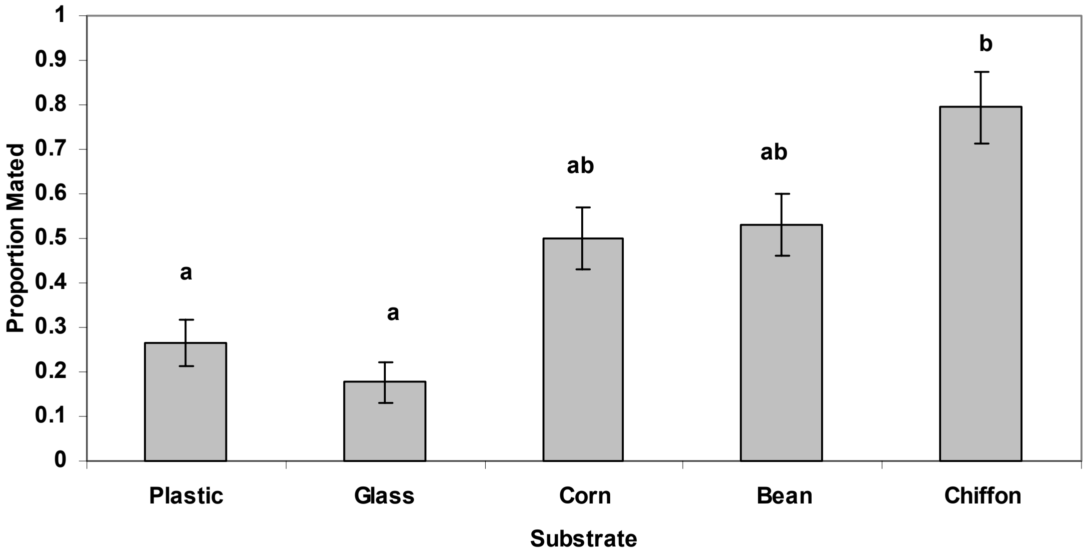

Fig. 3.2. The proportion of *C. marginiventris* females successfully mating on each of five substrates (n=34 pairs/substrate). Different letters above columns indicate significant (*P*< 0.05) differences according to a Chi-square test, and Tukey-type comparisons for proportions.

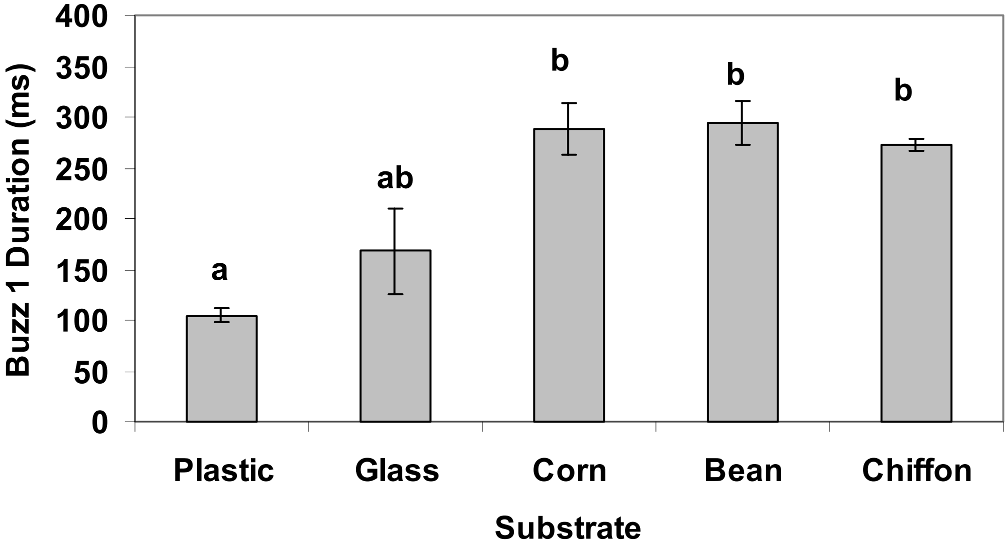

Fig. 3.3. The duration of the buzz 1 component of the courtship bout produced by male *C. marginiventris* on each of five substrates. Different letters above columns indicate significant differences (*P*<0.05) by a Kruskal-Wallis test.

Fundamental frequencies did not differ significantly between the beginning and end of buzz 1 for plastic or glass (Wilcoxon signed-ranks test, plastic, n=10, $P = 0.11$; glass, n=11, $P = 0.47$), but on corn, bean and chiffon, they were significantly lower at the end than at the beginning of the buzz 1 (Wilcoxon, corn, n=9, $P = 0.01$, bean, n=9, $P = 0.02$, chiffon, n=11, $P = 0.003$) (Fig. 3.4a).

The frequency of the second harmonic at the beginning of buzz 1 did not differ significantly among the various substrates (ANOVA, $F_{4, 45} = 1.72$, $P = 0.16$) (Fig. 3.4b). However, at the end of the buzz 1, the frequency of the second harmonic was lower on corn, bean and chiffon relative to plastic, and was lowest on bean (Kruskal-Wallis, $\chi_4^2 = 33.49$, $P < 0.001$) (Fig. 3.4b). Except in plastic (Wilcoxon, n=10, $P = 0.06$), all other substrates had significantly lower second harmonic frequencies by the end relative to the beginning of buzz 1 (Wilcoxon, glass, n= 11, $P = 0.01$; corn, n=9, $P = 0.01$; bean, n= 9, $P = 0.01$; chiffon, n= 11, $P = 0.003$) (Fig. 3.4b).

The frequency of the third harmonic at the beginning of buzz 1 did not differ among substrates (ANOVA, $F_{4, 45} = 1.29$, $P = 0.29$) (Fig. 3.4c). However, at the end of buzz 1, third harmonic frequencies were lower on corn, bean, and chiffon, than on plastic and glass (ANOVA, $F_{4, 44} = 12.75$, $P < 0.001$) (Fig. 3.4c). Comparison within a substrate at the beginning of buzz 1 relative to the end of buzz 1, found no significant change on plastic (Wilcoxon, n=10, $P = 0.44$), but the other substrates had lower

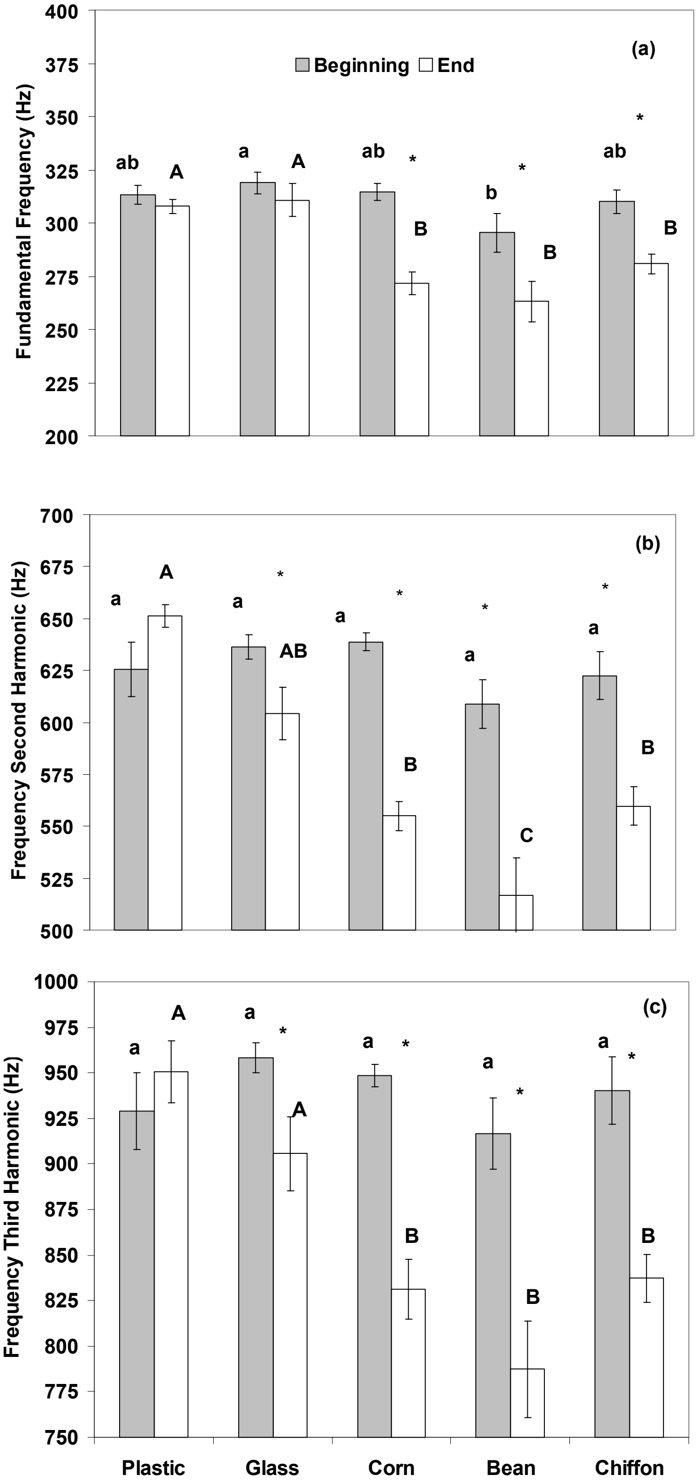

Fig. 3.4. The frequencies of the fundamental (a), second (b), and third (c) harmonic at the beginning and end of buzz 1. Among substrates, lower-case letters indicate significant differences at the beginning of buzz 1, upper-case letters indicate differences at the end of buzz 1, and asterisks indicate significance within a substrate ($P \leq 0.05$).

frequencies at the end of buzz 1 relative to the beginning of buzz 1 (Wilcoxon, glass, n= 11, $P = 0.01$; corn, n=9, $P = 0.01$; bean, n=9, $P = 0.01$; chiffon, n=11, $P = 0.003$, respectively.).

At the beginning of buzz 1, the amplitude of the fundamental frequency was higher on chiffon than plastic, glass and bean (ANOVA, $F_{4,45} = 7.43$, $P < 0.001$) (Fig. 3.5a). At the end of buzz 1, the relative amplitude was highest on chiffon (ANOVA, $F_{4,45} = 13.95$, $P < 0.001$) (Fig. 3.5a). The amplitude change of the fundamental frequency between the beginning of buzz 1 relative to the end of buzz 1 was not significant on most substrates (*t*-test, plastic, t_9=1.93, $P = 0.09$; corn, t_8 =1.36, $P = 0.21$; bean, $t_8 = 0.12$, $P = 0.91$; chiffon, $t_{10} = -1.01$, P =0.30; *t*-test, $t_{10} = 4.89$, $P = 0.001$) (Fig. 3.5a).

Second harmonic amplitudes at the beginning of buzz 1 differed among the substrates (ANOVA, $F_{4,45} = 16.54$, $P < 0.001$), chiffon having the greatest amplitude and plastic having the lowest (Fig. 3.5b). At the end of buzz 1, relative amplitude was higher on chiffon than other substrates (ANOVA, $F_{4,45} = 9.21$, $P < 0.001$). All substrates had a significant decrease in amplitude from the beginning to the end of buzz 1 (*t*-test, plastic, $t_9 = 2.80$, $P = 0.02$; glass, t_{10} =4.28, $P = 0.002$; corn, t_8=5.68, P<0.001; bean, t_8=3.32, $P = 0.01$; chiffon, t_{10}=3.88, $P = 0.003$) (Fig. 3.5b).

The third harmonic amplitude was highest for chiffon at the beginning of buzz 1 (ANOVA, $F_{4,45}$=9.32, $P < 0.001$), as well as at the end of buzz 1 (ANOVA, $F_{4,44} = 7.50$, $P < 0.001$) (Fig. 3.5c).

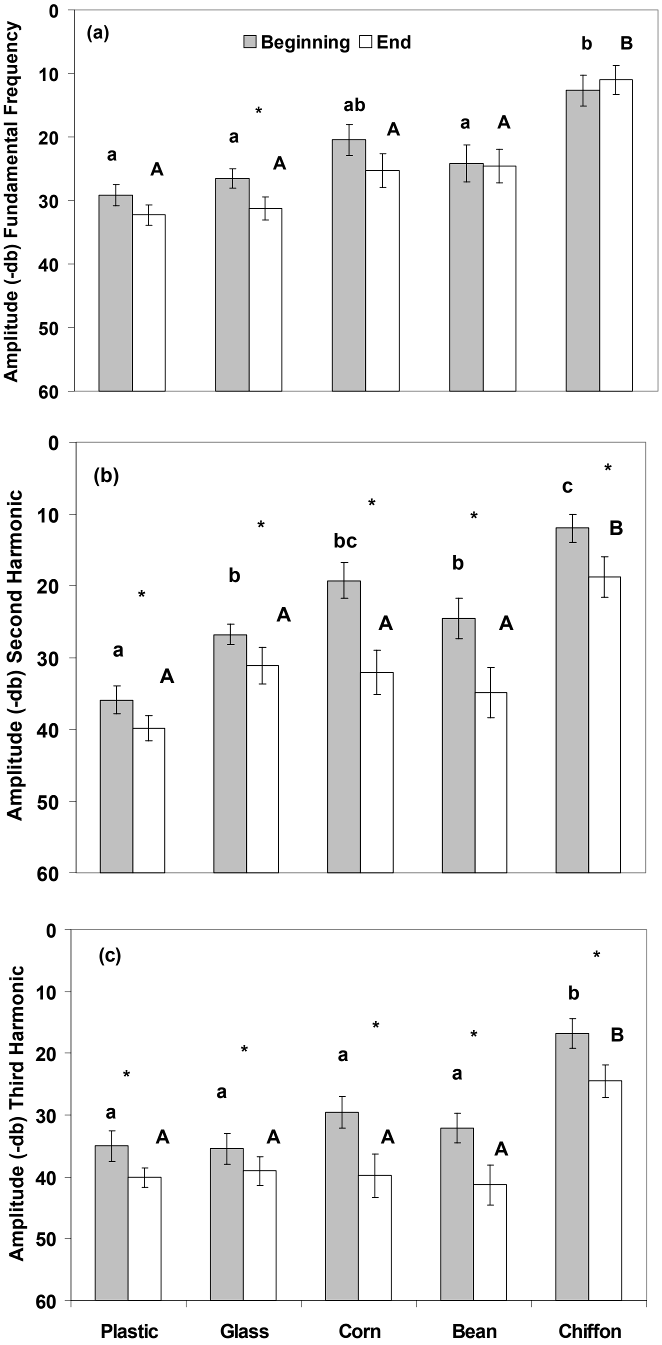

Fig. 3.5. The relative amplitude of the fundamental (a), second (b), and third (c) harmonic at the beginning and end of the buzz 1 component. Lower-case letters indicate significant differences among substrates at the beginning of buzz 1, upper-case letters indicate differences at the end of buzz 1, and asterisks indicate a significant difference within a substrate between the beginning and end of buzz 1 ($P \leq 0.05$).

All substrates had significantly decreases in amplitudes from the beginning to the end of buzz 1 (*t*-test, plastic, t_9=2.39, *P* = 0.04; glass, t_{10} =3.54, *P* =0.006; corn, t_8=3.08, *P* = 0.02; bean, t_8=4.07, *P* = 0.004; chiffon, t_{10}=3.59, *P* = 0.005) (Fig. 3.5c).

At the beginning of buzz 1, the ratios of the relative amplitudes of the first and second harmonic did not differ significantly among substrates (KW, 2.23, *P* = 0.69) (Fig. 3.6a). At the end of buzz 1, there were differences in the ratios of harmonics 1 to 2 (KW, 11.07, *P* = 0.03 (Fig. 3.6a), with the ratio for chiffon lower than plastic or glass (Fig. 3.6a), indicating that the first harmonic was about twice as loud as the second harmonic on chiffon, while the first and second harmonics on plastic and glass were of similar amplitudes. Comparisons within a substrate of the ratios of harmonic 1 to harmonic 2 between the beginning and end of buzz 1 were only different on chiffon (Wilcoxon signed-rank test, n=11, *P* = 0.05), and not other substrates (Wilcoxon, plastic, n =10, *P* = 0.80; glass, n= 11, *P* = 0.79; corn, n=9, *P* = 0.44; bean, n=9, *P* = 0.14) (Fig. 3.6a).

The ratio of harmonic 1 to 3 did not differ at the beginning of buzz 1 (KW, 2.66, *P* = 0.62), but there were differences in this ratio at the end of buzz 1 (KW, 16.34, *P* = 0.003) (Fig. 3.6b). The ratio of harmonic 1 to 3 was lower on chiffon and bean than on plastic, glass or corn, again indicating that the first harmonic was louder than the third on both chiffon and bean, while harmonics 1 and 3 on plastic, glass or corn had similar volumes (Fig.3.6b). Comparison within a substrate of the ratio of harmonic 1 to 3 between the beginning and end of buzz 1 were not significantly different for most

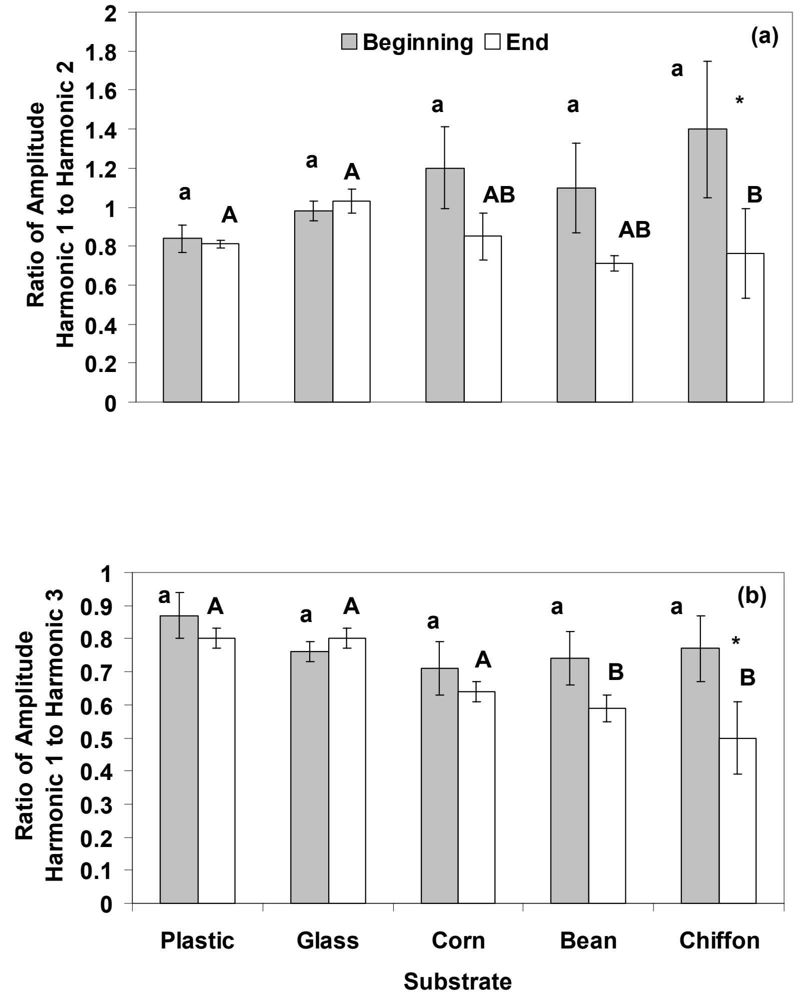

Fig. 3.6. The ratio of the relative amplitude of harmonic 1 to harmonic 2 (a), and harmonic 1 to harmonic 3 (b), at the beginning and end of buzz 1. Lower case letters indicate significant differences among substrates at the beginning of buzz 1, upper-case letters indicate differences at the end of buzz 1, and asterisks indicate a significant difference within a substrate between the beginning and end of buzz 1 ($P \leq 0.05$).

substrates (Wilcoxon, plastic, n =10, *P* = 0.80; glass, n= 11, *P* = 0.39; corn, n=9, *P* = 0.37; bean, n=9, *P* = 0.07), except on chiffon (Wilcoxon, n=11, *P* = 0.006) (Fig. 3.6b).

Role of Airborne and Substrate Vibration in Mating

Both the mating substrate and the presence of wings significantly predicted the probability of mating (Table 3.1). The odds of mating were 4.3 times greater on chiffon than on glass, and 9.6 times greater for normal versus dealated males (Fig. 3.7, Table 3.1).

After wing ablation, both dealated and normal males courted and produced detectable substrate vibrations (Fig. 3.8, 3.9). Mean velocities produced by dealated males were 72% lower after ablation (2.58 mm/s before, 0.72 mm/s after) (two-tailed, *t*=3.86, *P* = 0.005, df=8) (Figs. 3.8a,b, Fig. 3.9), while a change in mean velocity was not detected in normal males after mock ablation (two-tailed, *t*=0.015, *P* = 0.988, df=7) (Fig. 3.8 c, d, Fig. 3.9).

Table 3.1. Logistic regression of probability of mating. Two predictor variables for substrate (glass = 0 and chiffon= 1) and two predictors for wings (winged = 0 or dealated = 1); Log likelihood = -36.068, LR Chi^{2}2=20.85, *P* = 0.000).

Predictor	Coefficient	Odds Ratio	Z	*P*
Substrate	1.45	4.29	2.4	0.000
Wings	2.26	9.63	3.56	0.000

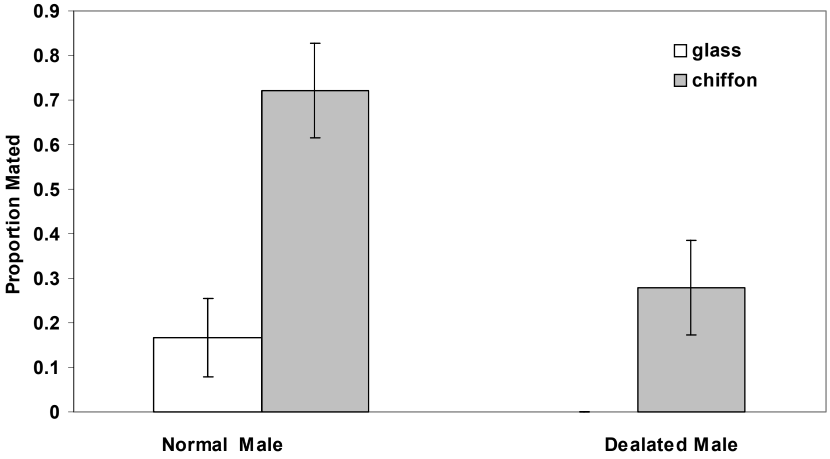

Fig. 3.7. The proportion (± S.E.) of normal and dealated *C. marginiventris* males mating on two substrates, glass and chiffon (n=18/category).

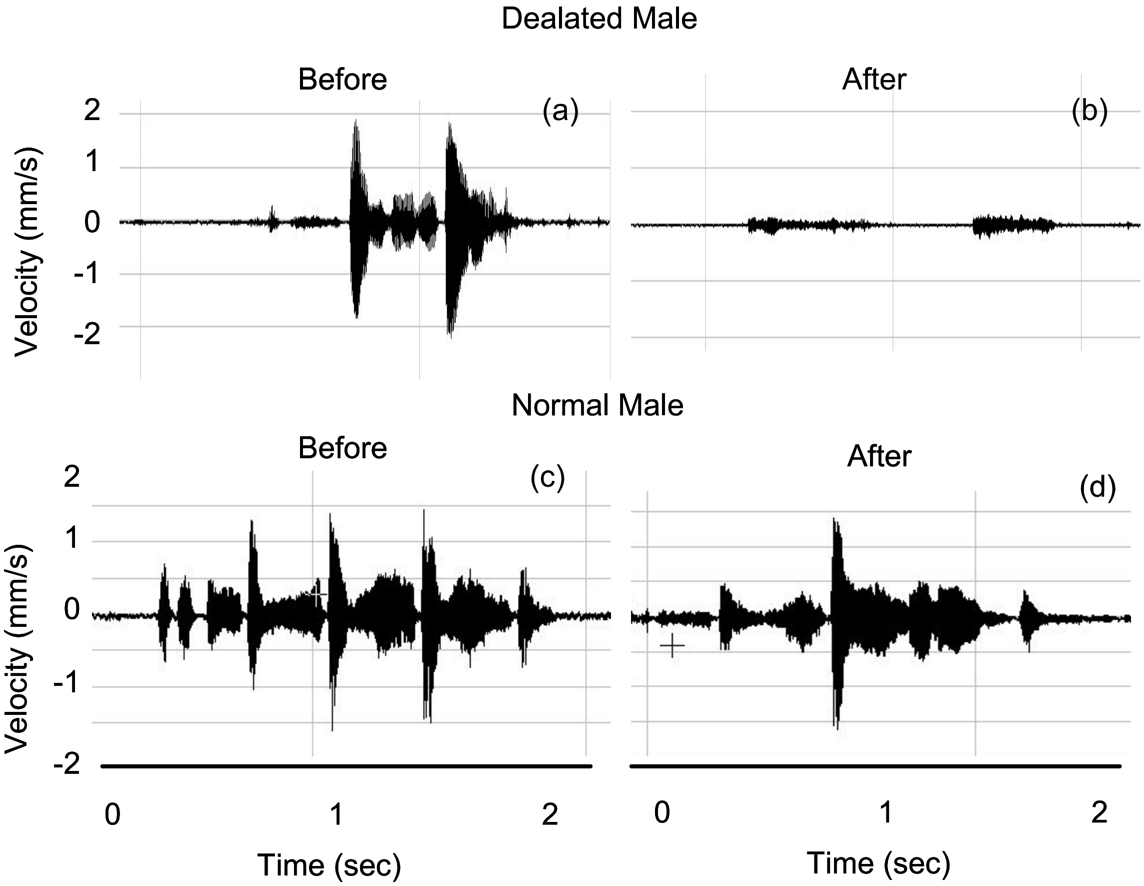

Fig. 3.8. The velocity of male *C. marginiventris* courtship vibrations on a chiffon arena for a dealated male before (a) and after (b) wing ablation, and a normal male before (c) and after (d) mock wing ablation.

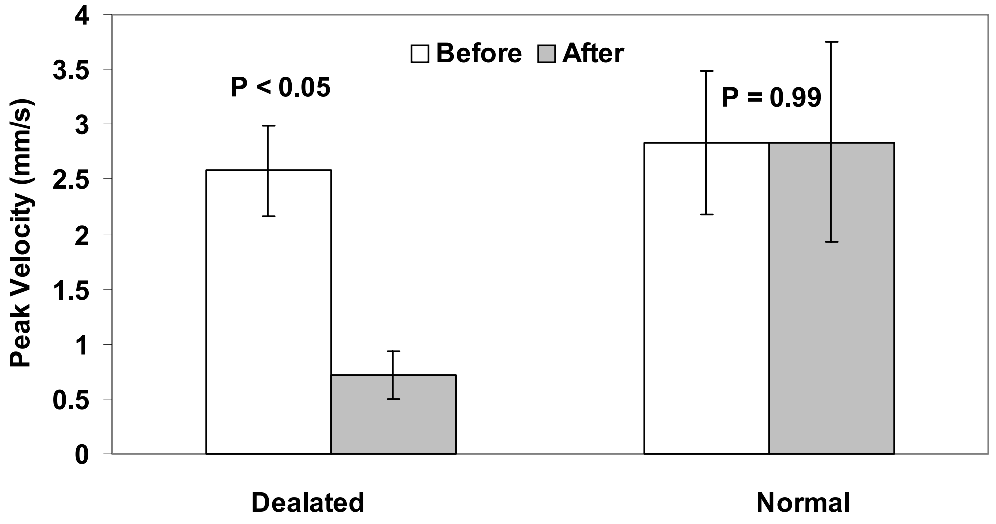

Fig. 3.9. The mean peak velocity (Mean ± S.E.) from the first courting bout of male *C. marginiventris*, for a dealated male prior to and after wing ablation, and a normal male prior to and after mock ablation. A paired *t*-test was conducted within the dealated and normal pairs, to test if there was a difference in courtship vibration amplitude after ablation or mock ablation.

Discussion

Mating Success on Five Substrates

The mating success of *C. marginiventris* was highest on chiffon, a rearing substrate, followed by the two host plants, bean and corn, and lowest on glass and plastic, both common rearing substrates. Greater mating success on host plants than on artificial substrates, such as glass and plastic, was expected because parasitoid males are more attracted to females in the presence of host plants (McAuslane *et al.*, 1990). However, mating success was similar on chiffon and the two host plants, which suggests that physical properties of the substrate are important for mating in *C. marginiventris*. Prior studies showed that vibrations transmit differently through common building materials (Rossing and Fletcher, 2004), and that physical properties of the mating substrate may affect mating rates in insects that use vibrational communication (Miklas *et al.*, 2001; Elias *et al.*, 2004; Henry and Martinez Wells, 2004; Cocroft *et al.,* 2006) .

Parasitoid sex ratios (percentage females) are frequently lower in laboratory cultures than in the field, which may be partially attributable to a high frequency of virgin females in laboratory cultures. For example, *C. marginiventris* sex ratios were lower in several laboratory studies relative to field studies (Novoa and Luna, 1996; Gillespie *et al*., 1997), as were the sex ratios of *Cotesia melanoscela* (Ratzeburg)*,* a parasitoid of the gypsy moth, and *Glyptapanteles militaris* (Walsh) (Braconidae), a parasitoid of *Pseudaletia unipuncta* (Haworth) (Kolodny-Hirsch, 1988; Kruse and Raffa, 1997; Oliveira *et al.*, 1999). In the case of *C. marginiventris* and *C. melanoscela,* it is plausible that a high frequency of virgin females, due to the absence of an appropriate mating substrate, could lead to lower sex ratios in the laboratory.

Transmission of Courtship Vibration on Five Substrates

The duration of buzz 1 was longer on corn, bean or chiffon than it was on plastic, and it was intermediate on glass (Fig. 3.3). Longer courtship vibration durations may increase the mating success for *C. marginiventris*, as demonstrated for other insects that use vibrational communication. Longer artificial courtship signals (600-800ms) played to *N. viridula* males elicited stronger responses relative to shorter signals (< 600 ms) (Miklas *et al.*, 2001). The courtship vibration duration of *C. marginiventris* males may be shorter on plastic or glass, relative to other substrates, because at their size male *C. marginiventris* may not produce sufficient energy during wing fanning to resonate these materials.

The mean frequencies of courtship vibrations were measured at the beginning and end of buzz 1 for each substrate (Fig. 3.4a-c), to examine how substrates filter the transmission of courtship vibrations. Filtering was more evident in corn, bean, and chiffon than in plastic and glass (Fig. 3.4a-c), which had lower frequencies at the end of buzz 1 relative to the beginning. Prior studies showed that leaves can act as low-pass filters (Casas and Magal, 2006), and may allow low frequencies to travel farther while filtering out higher frequencies. Vibrational signals may be produced with broad frequency ranges to increase the likelihood of successful transmission through vegetation, and each plant may filter signals differently (Michelsen *et al.*, 1982).

Cotesia marginiventris mated more frequently on corn, bean, and chiffon (Fig. 3.2), so the frequency modulation of buzz 1, between the beginning and end of buzz 1, may be an element of the courtship vibration that *C. marginiventris* can detect.

There were few differences in signal transmission characteristics through the two host plants that were evaluated. There was no difference in the duration of buzz 1 on bean or corn (Fig. 3.3), and relative amplitudes of the vibrations were similar in bean and corn for all harmonics (Fig. 3.5). Cocroft *et al.* (2006) recorded courtship vibrations of the treehopper *U. crassicornis* on a woody host and a woody non-host plant, and found no significant differences in signal parameters between the two plants. However, there was an effect of distance on the signal transmission, and by 10 cm there were differences in the final frequency and the signal duration. Similar observations were made for courtship vibrations of the lacewings *C. downesi* and *C. plorabunda*, which attenuated by a distance of 20 cm (Henry and Martinez Wells, 2004). In this study, the distances from recordings were < 2 cm, thus the effect of distance on amplitude was likely negligible.

The relative amplitudes of the courtship signals in all five substrates for the fundamental frequency (~300 Hz), second harmonic (~600 Hz) and third harmonics (~900 Hz) were greatest on chiffon followed by corn, and overall chiffon carried the highest amplitude for the first three harmonics (Fig. 3.5a-c). Chiffon and corn were the thinnest substrates among those used, so likely the most elastic and best able to transmit vibrations. As noted by others, thick substrates more than thin ones can decrease the amplitude of a vibratory signal (Cocroft *et al.,* 2006).

Other studies involving arthropods showed that substrates influence the transmission of vibrational signals used in courtship and foraging. For example, rock attenuated (decreased) the volume of all frequencies in the courtship signal of the spider *H. dossenus*, while frequencies passed through leaf litter without significant attenuation (Elias *et al.*, 2004). Moreover, sand acted as a band-pass filter, allowing frequencies of ~1000 Hz to pass through with much greater amplitude than lower frequencies, and filtered out lower frequencies that were important for communication (Elias *et al.*, 2004). Host location in parasitoid wasps that use vibrational sounding, and drum their antennae to locate concealed hosts, is also influenced by the substrate. Substrate density influenced the ability of two parasitoid wasps, *Pimpla turionellae* L. and *Xanthopimpla stemmator* (Thunberg), to locate their hosts (Fischer *et al.*, 2003), perhaps due to decreasing signal volumes with substrate density. Substrate also influenced transmission of honey bee waggle dance vibrations, which are associated with foraging. Transmission of artificial vibrations of 250 Hz, examined in honeybee combs made of beeswax and artificial plastic combs, had greater amplitude in beeswax, and was highly dependent on the substrate (Seeley *et al.*, 2005).

Role of Airborne and Substrate Vibration in Mating

The mating success of dealated males was lower than that of normal males on both glass and chiffon substrates. Both wings and substrate were important to mating success, which suggests that female *C. marginventris* responded to both near-field sound and substrate vibration. Wing fanning by males during courtship produced both substrate vibration and near-field sound, and Hymenoptera can detect near-field sound (airborne particle velocity) (Towne and Kirchner, 1989) and substrate vibrations (Meyhöfer and Casas, 1999; Tomov *et al.*, 2003). Attempts to separate the roles of the airborne particle velocity and substrate vibration potentially used by parasitoids as courtship signals have had mixed success (Ruther *et al.*, 2000). Other studies found that mating success was lower in dealated parasitoid males (Miller and Tsao, 1974; Kitano, 1975; Rungrojwanich and Walter, 2000), but the presence of wings appeared more important for some species than others (van den Assem and Putters, 1980; Ruther *et al.*, 2000). The details of the wing ablation procedure, and the substrates used in previous studies were not available in every case, so comparisons among those studies are not possible. If near-field sound was the primary courtship signal used by *C. marginiventris*, then substrate should have weakly influenced mating success. However, the substrate strongly influenced the mating success of both dealated and normal males; both dealated and normal males mated more frequently on chiffon than glass. Thus, *C. marginiventris* may detect the airborne near-field sound component of courtship as well as substrate vibrations. A prior study of *Cotesia rubecula* (Marshall) showed that substrate vibrations are essential for mating (Field and Keller, 1993a). Vibrational communication during courtship appears important as well for *C. marginiventris*, and could by used by other species of parasitoids in the family Braconidae, or by other parasitoids known to produce species specific patterns of vibration (Leonard and Ringo, 1978; van den Assem and Putters, 1980; Sivinski and Webb, 1989).

Courtship communication via substrate vibration in *C. marginiventris* would be demonstrated if females engaged in a vibrational duet with a male, as commonly occurs in other insect groups such as Hemiptera (Hunt *et al.*, 1992; Čokl and Virant Doberlet, 2003). Female *C. marginiventris* in this study did not respond to males with a duet, nor did they change their posture, as observed for female *C. rubecula* (Field and Keller, 1993a). In this study, however, females responded to male courtship by accepting courting males with frequencies that varied among the mating substrates that were evaluated. Other courtship signals, such as visual and chemical signals, may be used by *C. marginiventris*, but it is not likely that substrate would influence these courtship signals and impact female mating success. Chemical cues, such as pheromones, would likely be similar among substrates, though may be synergized by the presence of a plant odor (McAuslane *et al.*, 1990). The highest mating frequencies were on chiffon, followed by the two host plants, and lowest on glass and plastic. Mating success on chiffon was significantly higher than on plastic or glass, indicating that physical properties of the substrate and/or physical cues such as courtship vibrations are important for mating success in *C. marginiventris*.

The amplitude (velocity) of courtship vibrations produced by *C. marginiventris* was in the range of vibrations produced and detected by other courting insects, suggesting that these parasitoids, or other insects they might encounter, could detect these vibrational signals. Vibrational courtship signals with velocities as low as 0.1 mm/s are considered in the range of 'hearing' for insects (Michelsen *et al.*, 1982). Courting male *C. marginiventris* had a mean peak velocity of 2.58 mm/s when courting on chiffon fabric, which was comparable to values of vibrational courtship signals from *N. viridula* males, which ranged from 2 to 3.5 mm/s (Miklas *et al.*, 2001).

Conclusion

The mating substrate influenced the mating success of *C. marginiventris*. In addition, courtship vibrations transmitted differentially through the various rearing substrates. Evidence was presented that *C. marginiventris* used vibrational signals during courtship, and that transmission of these signals can be impacted by various substrates associated with mass rearing. Knowledge of transmission characteristics for courtship vibrations of substrates used in rearing could be important for other *Cotesia* used for biological control, or species used as model systems in behavioral, physiological and evolutionary studies (Michel-Salzat and Whitfield, 2004). Substrate vibrations are likely important to parasitoids in other families as well.

Informed selection of materials used for construction of rearing cages or containers could increase the mating rates of parasitoids that use vibrational communication. Cages constructed with at least one side of fabric may allow transmission of courtship vibrations, but cages consisting entirely of plastic, wood or glass, might prevent adequate transmission of courtship vibrations. A higher frequency of mated females should result in a greater proportion of female to male offspring, a factor important to rearing parasitoids for biological control. A study at a scale larger than this study could elucidate the importance of interacting factors affecting mating success and offspring sex ratios, such as cage construction materials, sex ratio distorting microorganisms, and genetic sex determination factors, all which are known to influence parasitoid sex ratios. Other orders of insects are known to use vibrational communication (Cocroft and Rodriguez, 2005), and knowledge of the transmission characteristics of their courtship signals through substrates could be important for effective rearing as well.

CHAPTER IV

MATE CHOICE IN A SOLITARY AND GREGARIOUS PARASITOID, *Cotesia marginiventris* AND *Cotesia flavipes*

Introduction

Mate choice has rarely been addressed in parasitoid wasps (Godfray and Cook, 1997; Quicke, 1997), perhaps due in part to the minute size of these insects. Parasitoid wasps oviposit and develop in other species of host insects, and are often used in biological control programs to suppress populations of insect pests. Females are generally predicted to be the selective sex when choosing mates, because they typically need to mate only once to fertilize their eggs (Thornhill and Alcock, 1983; Davies, 1991; O'Neil, 2001), and thus may benefit from assessing mate quality. Parasitoid males are not known to offer females direct material benefits or resources, such as spermatophores or nuptial gifts, to entice females to mate (Godfray, 1994), but female parasitoids could receive indirect, genetic or fitness benefits from mates, as shown in other insects (Legner, 1989; Capone, 1995; Greenfield, 2002).

Mate choice may be influenced by the mating system, which often reflects the spatial location of hosts in the field (Thornhill and Alcock, 1983; Davies, 1991; Godfray and Cook, 1997). Godfray (1994) proposed a classification of parasitoid mating systems based on an ecological framework. Males and females emerging in different parts of the environment, such as solitary parasitoids, must search for mates, and males may compete to find females (Godfray, 1994). When males or females are concentrated in the environment, such as for gregarious parasitoids, males may compete directly for mates

(Hamilton, 1967; Hardy, 1994; Godfray and Cook, 1997). Gregarious parasitoids are predicted to exhibit a greater level of male competition for mates than solitary parasitoids. Similarly, male competition for mates is predicted in other insect systems where resources including females are concentrated (Thornhill and Alcock, 1983; Davies, 1991). No predictions of female choice were made for either of the above two mating systems.

Few studies have directly examined female or male mate choice in parasitoids (Ode *et al.*, 1995; Godfray and Cook, 1997; Gu and Dorn, 2003). Females may appear to choose the larger mate, but he may be the winner of a male competition prior to a mating attempt with a female. In both solitary and gregarious parasitoids, a large-male advantage in mate acquisition has been documented (Eggleton, 1990; Lampson *et al.,* 1996; Abe *et al.*, 2005), and alternative mating tactics, such as mate stealing, have been observed (Field and Keller, 1993b). In contrast, several studies found no large-male precedence or advantage in mating (Crankshaw and Mathews, 1981; Suzuki and Hiehata, 1985; Antolin and Strand, 1992; Cheng *et al.*, 2003). Studies in the laboratory and the field may provide useful insight to mate choice processes (Godfray and Cook, 1997).

The goal of this study was to test whether female or male choice for large or small mates occurs in two parasitoids with putatively different mating systems, the solitary parasitoid, *Cotesia marginiventris* (Cresson), and the gregarious parasitoid, *Cotesia flavipes* Cameron. Once males are in close proximity to females, males begin wing fanning and courting females. Mate choice was examined at the courtship level, once males and females were in close proximity to each other. Additionally, precopulatory behaviors of males were examined for evidence of male competition, or to determine if there was a large-male advantage in mate acquisition. Male competition is predicted to be more likely in the gregarious parasitoid, *C. flavipes*, than in the solitary parasitoid, *C. marginiventris*.

Materials and Methods

Study Species

The first parasitoid, *Cotesia marginiventris*, is a solitary endoparasitoid (Boling and Pitre, 1970; Tillman, 2001), and males and females search to locate mates. Male development time is one day shorter than female development time, and males are attracted to a female pheromone on the first day of adult life (A. J., unpubl. data). Mean adult lifespan of females is 4-9 d at 27 °C (Kunnalaca and Mueller, 1979).

The second parasitoid, *Cotesia flavipes*, is gregarious, and at least a partially locally mating species. Broods of ~ 40 individuals emerge from a single host, mostly within one hour, and typically have a female-biased sex ratio (Wiedenmann *et al.*, 1992; Kimani and Overholt, 1995; Potting *et al.*, 1997). Males mate with multiple females, but females appear to mate only once (Arakaki and Ganaha, 1986). Mean adult lifespan is 2-5 d in the laboratory (Wiedenman *et al.*, 1992; Potting *et al.*, 1997).

The courtship behaviors of *C. flavipes* and *C. marginiventris* are similar (Kimani and Overholt, 1995; Chapter II). Once the male is within several cm of the female, he detects a female sex pheromone and fans his wings. Males then approach females and attempt to copulate. Receptive females remain stationary and lower their body to the substrate, and often elevate the wings, or they reject males by jumping away while fanning their wings (A. J., unpubl. data). Both of these *Cotesia* species are several mm in size, and there are no apparent color patterns or markings on the wasps that might provide visual cues or signals to a potential mate.

Rearing Insects

Cotesia marginiventris was reared using larvae of the moth *Spodoptera frugiperda* (J. E. Smith) as hosts, and *C. flavipes* was reared using larvae of *Diatraea saccharalis* (F.) (Chapter II). Before experiments, parasitoid cocoons were collected from the plastic cups containing host larvae, and placed singly in 1 ml (¼ dram) vials with a streak of honey so that emerging adults would remain virgin until used in an experiment.

Plants

Trials involving *C. marginiventris* (the solitary species) were conducted in an arena positioned on a leaf of a young maize plant (*Zea mays* L.). Preliminary trials showed that a low proportion (< 30%) of this parasitoid species mated when placed together in glass vials or in plastic Petri dishes, and that using a plant as a mating substrate substantially increased this proportion to ~ 75% (see Chapter III).

Maize plants for the *C. marginiventris* trials (below) were grown from Pioneer® seed (34A55) (Johnston, Iowa, U.S.A.), planted in Miracle Grow® (Marysville, Ohio, U.S.A.) potting soil in green plastic pots (13 cm diam × 12 cm tall) in a greenhouse with natural light at 15L:10D, temperature at 30 °C ± 5 °C, and 50-90% RH. Plants used in experiments were 30-40 cm tall, and had 5 leaves.

Selecting Large and Small Parasitoids

Each trial used newly emerged, virgin adult male and female parasitoids. A droplet of honey was placed on the inside edge of each vial containing an adult parasitoid so it would pause to feed temporarily, and its size could be assessed. A binocular microscope fitted with a lens micrometer was used to grossly estimate body length, and parasitoids were grouped into "small" or "large" size classes. The difference in large or small wasps was visually apparent to the unaided eye, and was later found to represent an approximately 10% difference in size between large and small males, as parasitoid right hind tibia were measured after the experiment. Parasitoid individuals from these large and small size classes were used in the mate choice trials described below.

Mate Choice Experiments

Trials involving *C. marginiventris* were conducted between 8:00 and 10:00 h in a laboratory, at 26 °C ± 1 °C, 50% ± 5% RH, with overhead fluorescent lights; preliminary trials showed that mating frequency decreased substantially after 10:00 h (A. J., unpubl. data). *Cotesia marginiventris* trials were conducted in an arena containing the leaf of a maize plant. Before setting up a mating arena on a maize plant, all leaflets were removed except the central whorl and one adjacent leaf. The lid of a 160 ml (40 dram) plastic vial with a 0.5 cm diam hole in it was placed over the center stem and an adjacent leaf, and, together with the vial served as an arena. Parasitoids, plants, and vials were used only once and then discarded.

Trials of *C. flavipes* were conducted in a laboratory at 24 °C ± 2 °C, 60% ± 2% RH, with overhead fluorescent lights, and some natural light available from windows. Trials with *C. flavipes* were conducted throughout the day, as they were observed to mate frequently during this time. *Cotesia flavipes* mating trials were all conducted in 24 ml (6 dram) glass vials (2.3 cm diam × 9 cm length), because preliminary trials showed that they mated at high frequencies (~ 80%) in glass vials (A. J., unpubl. data). Each parasitoid and vial was used only once.

Experiment 1: Mate Choice by* C. marginiventris *Females

Each trial was conducted in a clean 160 ml (40 dram) plastic vial arena on the leaf of a maize plant, as described above. All *C. marginiventris* used in these studies were less than 48 h old. Two males, one large and one small, were placed in the arena, and a female was subsequently placed into the same arena and onto the maize plant. Male precopulatory behaviors were recorded to determine if there was direct male-male competition or a large-male advantage. The frequency and the latency (time from start of experiment to first exhibition of a behavior) in seconds of the following male precopulatory behaviors were recorded for the large and small male in each trial: (i) first wing fanning, (ii) first approach to a female, and (iii) first attempted copulation. These were the only male precopulatory behaviors that were observed. Copulation duration was recorded, as well as the time elapsed from the start of the experiment until copulation began (hereafter "copulation latency"). A visual assessment was made to determine whether the large or small male mated first with the female. After mating was complete (or 15 min if no mating occurred), the mating pair was collected together into a vial, and the unmated individual was collected into a separate vial. A total of 43 trials were conducted, and females did not mate with either the large or small male in 14 trials. Following each trial, adults were killed by freezing, and the length of the right hind tibia was measured (mm) for both males and the female using a micrometer mounted on a binocular microscope. In all experiments, the right hind tibia length (RHTL) was used as a proxy for size.

Experiment 2: Mate Choice by **C. marginiventris** *Males*

Vial arenas and maize plants, as described above, were used in these trials. For each trial, a large and a small female were placed in the vial arena on a maize plant, followed by a male. The precopulatory behaviors of males described above were recorded as they were displayed toward the large or small females. In this experiment, the male precopulatory behaviors toward females were used as a measure of male preference. Copulation durations, and whether the large or small female was mated were recorded. After mating, or at the end of the trial (15 min), the mated pair was collected together in a vial, and the unmated individual was collected separately. A total of 26 trials were conducted, and the male did not mate with either the large or small female in 7 trials. The RHTL was measured in all parasitoids, as described above.

Experiments 3 and 4: Mate Choice by* C. flavipes *Females and Males

The trials with *Cotesia flavipes* were conducted as described above for *C. marginiventris*, with the exception that trials were conducted in 24 ml glass vials. *Cotesia flavipes* measure only several mm in size, so the arenas were extremely large in comparison to the size of the wasps. All *C. flavipes* used in these trials were less than 24 h old. In the female choice experiment, male precopulatory behaviors were recorded to determine if there was a large-male advantage or male-male competition. In the male choice experiment, male precopulatory behaviors toward large and small females were recorded, with the exception of wing fanning. At least 25 trials were conducted for each experiment, and all trials lasted 15 minutes. In the female choice experiment (Experiment 3), the female did not mate in 9 of 36 trials. In the male choice experiment (Experiment 4), the male did not mate in 5 of 25 trials. The RHTL was measured in all parasitoids as described above.

Statistical Analysis

Data from trials in which mating did not occur were excluded from all analyses. Mate choice data in the female and male choice experiments were analyzed via Chi-square tests, and compared the frequencies of mating or not mating for large or small individuals against an expectation that mating frequencies occurred independently of size. Size differences in the right hind tibia lengths between mated and unmated males (or females), were compared using *t*-tests. Additionally, paired *t*-tests were used to compare the mean size differences in the mated male and female RHTL, as well as between the unmated male and female size, for all the trials in each experiment. The goal was to examine whether mated males were larger in size relative to female size, and if unmated males were smaller in size than females. All *t*-tests were two tailed.

Mean copulation durations, as well as mean copulation latencies, were compared between large and small males in the female choice experiments. In the male choice experiments, these behaviors were compared between males that mated with large or small females. In the *C. flavipes* male choice experiment, only 1 male mated with a large female, so copulation duration and latency could not be compared. A Kolmogorov-Smirnov test was used to determine if each pair had similar distributions (e.g. between large and small males), and since the majority of distributions were different, Mann-Whitney U tests were used for comparisons between the two categories. When a category contained more than 10 observations, a large-sample Mann-Whitney U test was conducted, with corrections for tied data, and results were reported as Z-scores (Siegel and Castellan, 1988).

The frequencies of male precopulatory behaviors were compared using Chi-square tests, to infer whether there was a large or small-male mating advantage, or male-male competition based on male size (female choice experiment), or if there was male preference to court large or small females (male choice experiment). The latency to each precopulatory behavior by males was compared between large and small males using Mann-Whitney U tests. Chi-square tests were performed to determine if the frequency of attempted copulations by large or small males resulted in an equal frequency of matings or rejections for each male size. A Fisher's exact test was performed when any frequencies were lower than 5. Finally, the mean copulation durations and copulation latencies were compared between the female choice and male choice experiments within each species using Mann-Whitney U tests. Statistical analyses were conducted using Statistix (2000) and SAS (1996).

Results

Mate Choice by C. marginiventris *Females*

Significantly more *C. marginiventris* females mated with large males than with small males ($P<0.001$) (Fig. 4.1a). The right hind tibia length (RHTL) of mated males was significantly larger than that of unmated males ($P = 0.006$) (Fig. 4.1b). The mean size difference in RHTLs between the mated or unmated males, relative to the female from the corresponding trial, was significant ($P = 0.04$) (Fig. 4.1c). Mean copulation durations were similar in large or small males (25.8 ± 1.1 vs. 25.0 ± 2.1 sec) (large sample Mann-Whitney U, Z=0.29, $P = 0.77$, N1=19, N2=3), as were the copulation latencies for large and small males (352.2 ± 55.6 vs. 368.0 ± 127.7 sec) (Z=0.84, N1=24, N2=5, $P = 0.80$).

Large and small males were equally likely to be the first to exhibit wing fanning towards a female ($P = 0.38$), while large males were more likely than small males to first approach females ($P = 0.01$), but they attempted copulation first with equal frequency as small males ($P = 0.59$) (Table 4.1). The mean latencies for each of these three behaviors were not significantly different between large and small males ($P \geq 0.50$, large sample Mann-Whitney U tests) (Table 4.1).

Female behavior toward male copulation attempts depended on whether males were large or small. Females more frequently accepted large males over small males as mates on the first attempted copulation ($P = 0.02$) (Fig. 4.2a).

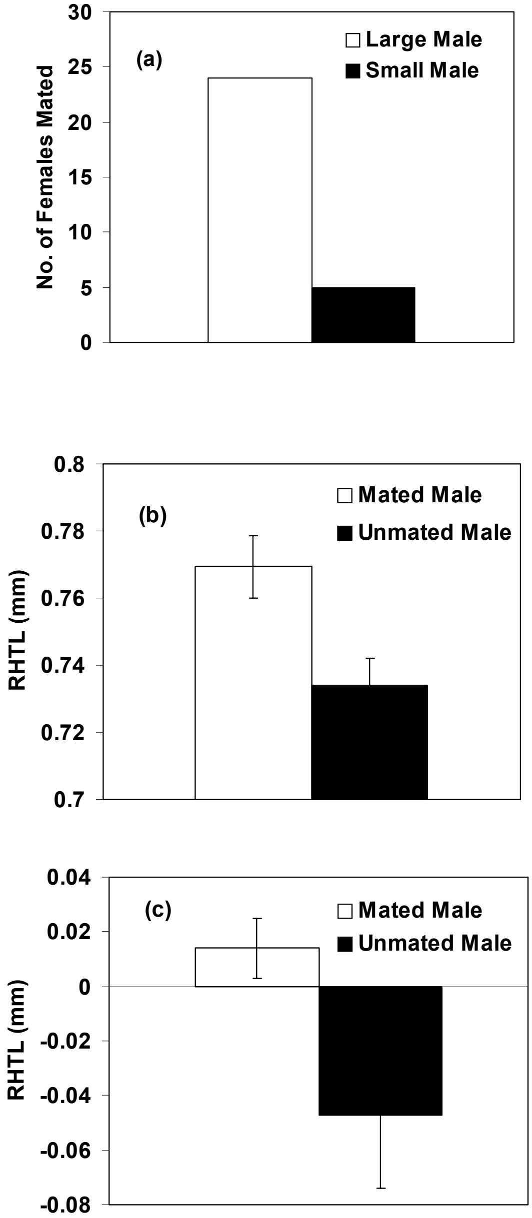

Fig. 4.1. Mate choice for large or small males by *Cotesia marginiventris* females. (a) Females mated more frequently with large than small males (Chi-square test: $\chi^2_1 = 22.35$, $P \leq 0.001$). (b) Mated males were larger than unmated males (t-test: $t_{54} = 2.91$, $P = 0.006$). (c) Mated and unmated male right hind tibia length (size) relative to females (Paired t-test: $t_{28} = 2.15$, $P = 0.04$).

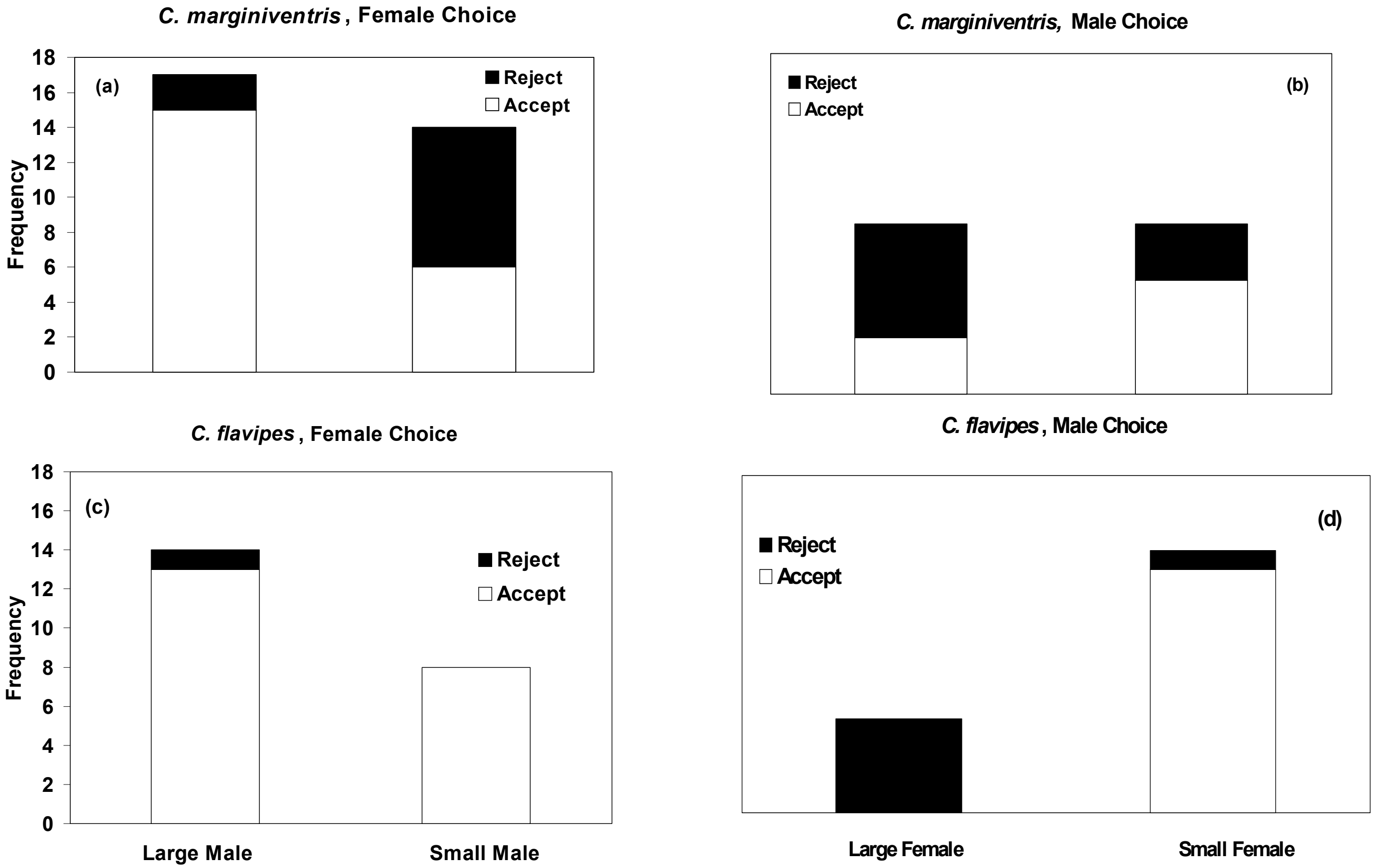

Fig. 4.2. Female rejecting or accepting the male on first attempted copulation. For *Cotesia marginiventris*, (a) large males were accepted more frequently as mates (Fisher's Exact Test, two-tailed, $P = 0.02$), although (b) the male choice experiment found no difference in acceptance or rejection based on female size (Fisher's Exact Test, two-tailed test, $P = 0.35$). For *Cotesia flavipes*, (c) large or small males were accepted by females with similar frequency (Fisher's Exact Test, two-tailed, $P < 0.001$), while (d) the male choice experiment found the first attempted copulation was accepted more frequently by small females (Fisher's Exact Test, two-tailed, $P = 0.001$)

Table 4.1. The frequency and latency to the first display of male precopulatory behaviors in the female and male choice experiments

	Male Precopulatory Behaviors					
	1st Wing Fan		1st Approach		1st Attempted Copulation	
C. marginiventris	First Display	Latency	First Display	Latency	First Display	Latency
(a) Female Choice	Frequency	Mean ± S. E. (s)	Frequency	Mean ± S. E. (s)	Frequency	Mean ± S. E. (s)
Large Male	13	40.2 ± 12.2	17	48.4 ± 15.1	15	192.9 ± 41.3
Small Male	10	55.9 ± 21.2	8	58.6 ± 22.1	13	129.9 ± 26.5
	χ^2=0.78, *P* =0.38	Z=0.62, *P* = 0.53	χ^2=6.83, *P* =0.01*	Z=0.38, *P* =0.70	χ^2=0.29, *P* =0.59	Z=0.68, *P* =0.50
(b) Male Choice						
Large Female	11	56.6 ± 17.9	11	59.6 ± 17.2	9	126.9 ± 30.0
Small Female	7	47.7 ± 23.1	8	44.1 ± 20.3	10	101.4 ± 22.3
	χ^2=1.78, *P* =0.18	Z=0.27, *P* = 0.79	χ^2=0.95, *P* =0.33	Z=0.91, *P* =0.36	χ^2=0.11, *P* =0.75	Z=0.57, *P* =0.57
C. flavipes						
(a) Large Male	7	32.0 ±15.7	10	18.7 ±2.2	14	37.8 ± 7.5
Small Male	17	10.0 ± 2.5	12	36.0 ± 11.1	10	63.8 ± 19.6
	χ^2=8.33,*P*=0.004	Z=2.048,*P*=0.04	χ^2=0.36, *P* =0.55	Z=0.50, *P* =0.62	χ^2=8.28, *P* =0.25	Z=0.53, *P* =0.60
(b) Large Female	n/a	n/a	8	21.1 ± 5.0	5	47.8 ± 12.2
Small Female	n/a	n/a	12	16.4 ± 2.7	15	52.0 ± 18.1
			χ^2=1.60, *P* =0.21	Z=0.50, *P* =0.62	χ^2=8.10,*P*=0.004	Z=0.87, *P* =0.38

In the female choice experiment, large or small males displayed toward a female. In the male choice experiment, the single male displayed toward a large or small female. First display frequencies were compared using a Chi-square test. The latency to first display of each behavior was compared using a Large Sample Mann-Whitney U test. (*= $P \leq 0.05$)

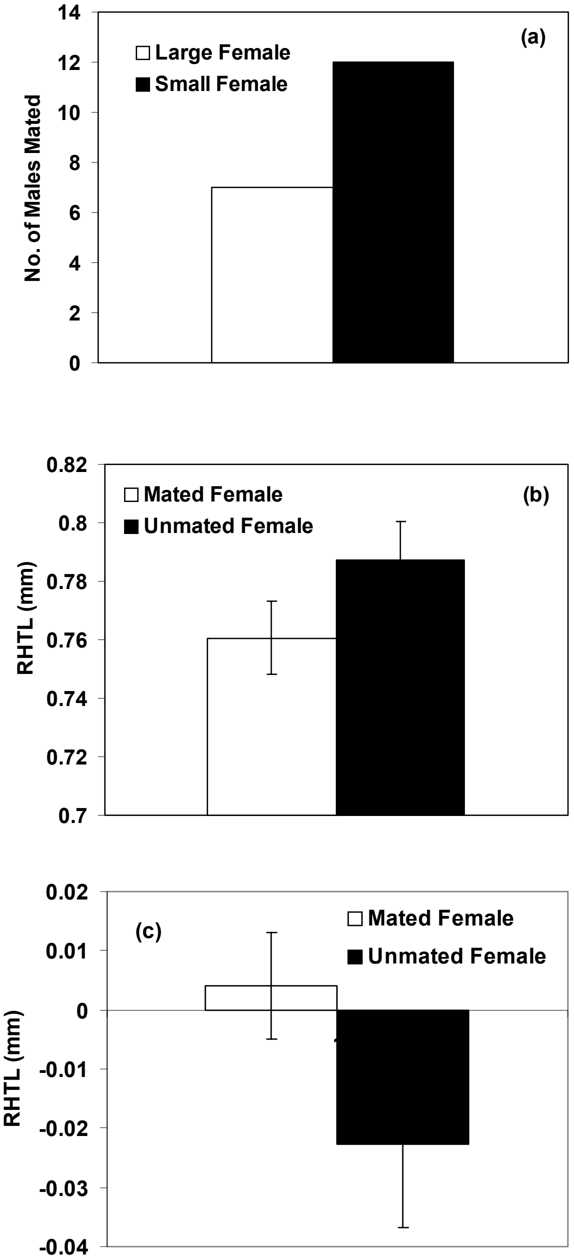

Fig. 4.3. Mate choice for large or small females by *Cotesia marginiventris* males. (a) Males mated with large or small females equally (Chi-square test: $\chi^2_1 = 2.63$, $P = 0.11$), (b) Mated and unmated females were similar sized (*t*-test: $t_{36} = 1.49$, $P = 0.07$), (c) Mated and unmated females had similar RHTLs relative to male size (paired *t*-test: $t_{19} = 1.99$, $P = 0.06$).

Mate Choice by C. marginiventris *Males*

The frequencies of male matings with large or small females were not significantly different (*P* = 0.11) (Fig. 4.3a). Mated and unmated females had similar RHTLs (*P* = 0.07) (Fig. 4.3b). The mean difference in RHTLs between the mated and unmated females relative to the males RHTLs were not significantly different (*P* = 0.06) (Fig. 4.3c). Copulation times did not differ between males mating with large or small females (23.5 ± 1.9 vs. 21.7 ± 1.3 sec) (Z= 1.02, N1=6, N2=11, *P* = 0.30).

Males fanned their wings with equal frequency towards large and small females (*P* = 0.18), approached large or small females with similar frequencies (*P* = 0.33), and attempted to copulate with similar frequencies the large or small females (*P* = 0.75) (Table 4.1). The latency to these three behaviors did not differ significantly between large and small females ($P \geq 0.36$, large sample Mann-Whitney U) (Table 4.1). Lastly, there was no significant difference in acceptance or rejection frequencies of males in the first copulation attempt by large or small females (*P* = 0.35) (Fig. 4.2b).

The copulation duration and the copulation latency were compared between the female choice experiment (where two males were present), and the male choice experiment (where only one male was present). The copulation duration was significantly longer when two males, rather than only one, were present (25.7 ± 1.0 vs. 22.4 ±1.1) (large sample Mann-Whitney U test, Z=2.08, N1=22, N2=17, *P* = 0.04), while the copulation latency was similar when one or two males were present (354.9 ± 50.1 vs. 322.7 ± 51.1 sec) (Z= 0.08, N1=29, N2=19, *P* = 0.93).

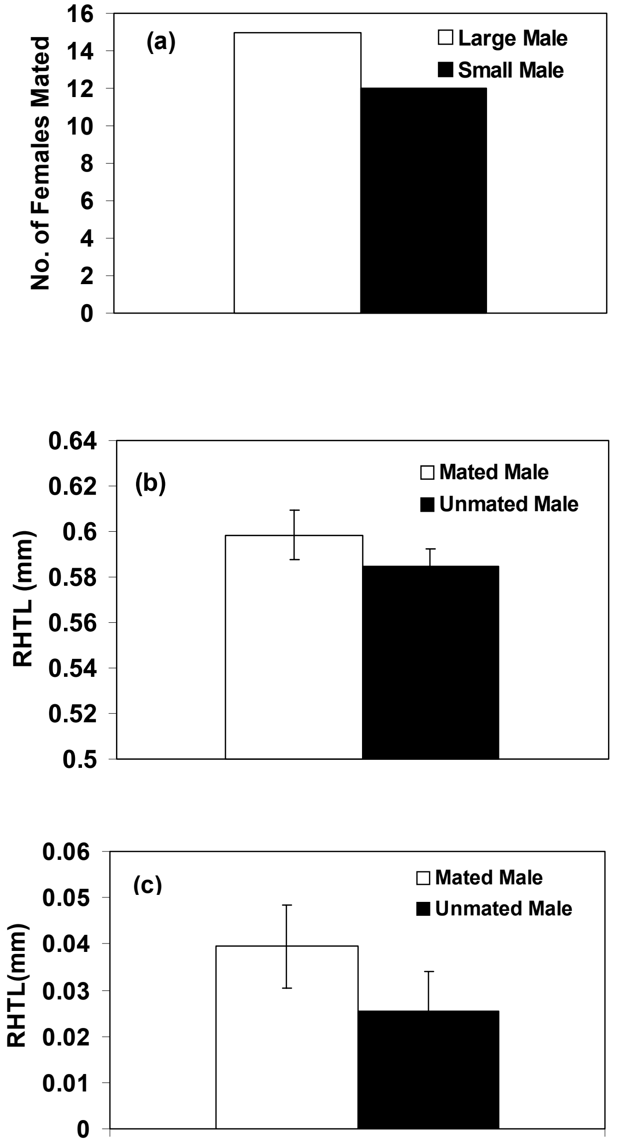

Fig. 4.4. Mate choice for large or small males by *Cotesia flavipes* females . (a) Females mated with large or small males with similar frequency (Chi-square test: χ^2_1 =0.41, $P = 0.41$). (b) Mated and unmated males were similar sized (*t*-test*:* $t_{48} = 1.02$, $P = 0.31$). (c) The RHTL size difference between mated males and females compared to unmated males and females was not different (paired *t*-test*:* t_{26} =1.16, $P = 0.26$).

Mate Choice by C. flavipes *Females*

Differences were not evident in the numbers of females mating with large or small males ($P = 0.41$) (Fig. 4.4a), or the size of mated or unmated males ($P = 0.31$) (Fig.4.4b). The mean difference in RHTLs between the mated males and females and unmated males and females were not significantly different ($P = 0.26$) (Fig. 4.4c). The mean copulation times of large and small males were not significantly different (20.2 ± 3.1 vs. 18.8 ± 2.4) (Z=0.49, $P = 0.63$, N1=15, N2=8), nor were the copulation latencies (128.0 ± 58.4 vs. 69.2 ± 20.4 sec) (large sample Mann-Whitney U, Z=0.21, N1=15, N2=9, $P = 0.84$).

Though small males were more likely than large males to first exhibit wing fanning behavior ($P = 0.004$), the frequencies of first approaches ($P = 0.55$) and first attempted copulations ($P = 0.25$) did not differ between large and small males (Table 4.1). Similarly, while the latency to wing fanning was significantly shorter for small males than large males (large sample Mann-Whitney U, $P = 0.04$), the mean latency to the first approach ($P = 0.62$) or attempted copulation ($P = 0.60$) did not differ significantly for large or small males (Table 4.1). Large males were as likely as small males to mate on the first attempted copulation ($P = 1.0$); however, practically all attempted copulations resulted in mating, and few (< 5%) females rejected mates (Fig. 4.2c).

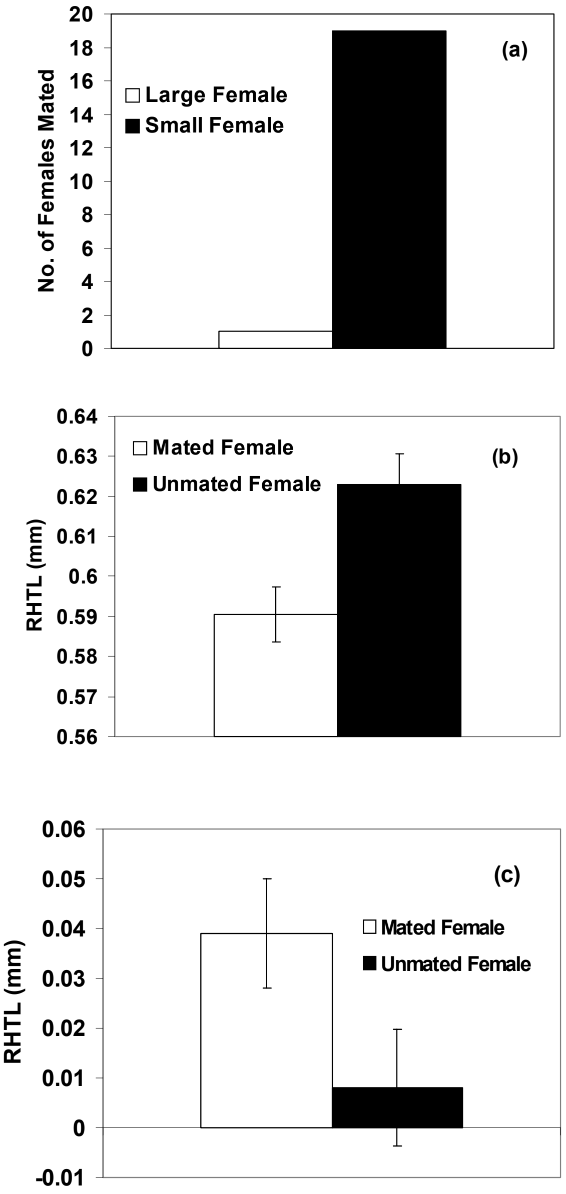

Fig. 4.5. Mate choice for large or small females by *Cotesia flavipes* males. (a) Males mated more frequently with small females (Chi-square test: $\chi^2_1 = 28.90$, $P < 0.001$). (b) Mated females were significantly smaller than unmated females (*t*-test: $t_{38} = 3.16$, $P < 0.001$). (c) There was a significant size difference between mated males and females compared to males and unmated females (paired *t*-test: $t_{20} = 4.68$, $P < 0.001$).

Mate Choice by **C. flavipes** *Males*

Males mated significantly more frequently with small females relative to large females ($P < 0.001$) (Fig. 4.5a), and mated females were significantly smaller than unmated females ($P < 0.001$) (Fig. 4.5b). The mean difference in RHTLs between mated females and males compared to unmated females and males was significant ($P < 0.001$) (Fig. 4.5c).

Males approached large or small females with similar frequencies ($P = 0.21$), but more frequently attempted copulation first with small relative to large females ($P = 0.004$) (Table 4.1), and first attempted copulations were successful more frequently with small than with large females ($P \leq 0.001$) (Fig. 4.2d). The latencies of these three behaviors towards large or small females did not differ significantly ($P \geq 0.38$, large sample Mann-Whitney U) (Table 4.1).

The mean copulation times in the female and male choice experiments of *C. flavipes* were not significantly different (19.8 ± 2.8 vs. 17.1 ± 1.0 sec) (large sample Mann-Whitney U, $Z = 1.10$, $N1 = 24$, $N2 = 20$, $P = 0.27$), nor were the copulation latencies (116.3 ±34.4 vs. 99.6 ± 23.4 s) (large sample Mann-Whitney U, $Z = 0.01$, $N1 = 27$, $N2 = 20$, $P = 0.99$).

Discussion

The results of the female choice trials involving the solitary species, *C. marginiventris*, suggested that females preferentially mated with the larger males (Figs. 4.1a-c). In contrast, the male choice trials for this species did not indicate a mate size preference. The difference between the size of potential male and female mating partners in the *C. marginiventris* female choice experiment was twice that in the male choice trials (0.06 vs. 0.03 mm, Figs. 4.1c, 4.3c). The greater size variation of males relative to females may provide females with more opportunity for selectively choosing their mates.

While *C. marginiventris* males are not known to offer female mates any direct benefit, female *C. marginiventris* mated more frequently with larger males in the female choice experiment. Female size preferences for male mates have been documented in insect systems where there is no apparent direct benefit of mate choice. For example, females of the green stink bug *Acrosternum hilare* (Say) (Pentatomidae) choose larger males as mating partners (Capone, 1995), though males do not provide parental care or resources, and it is not known whether mating with larger males is advantageous. Male and female parasitoid size may be heritable (Ellers *et al.*, 2001), though it can be influenced by host size (Charnov *et al.*, 1981; Joyce *et al.*, 2002; Cheng *et al.*, 2003). If male size is heritable, mating with large males may produce male offspring that could search a larger area for mates, out compete smaller males in mate location, or live longer. Larger male parasitoids typically live longer than smaller males (Bernal *et al.*, 2001; Sagarra *et al.*, 2001), and may have higher fitness than small males (Kazmer and Luck, 1995). The present study examined mate choice at the courtship level. Once males were courting females in the present study, females mated preferentially with the larger male when offered a choice of mate size.

The results of the *C. marginiventris* female choice experiment suggested that large males have an advantage over small males during courtship, because large males approached females first more frequently than small males. Larger parasitoids may disperse farther or search more area to locate hosts or potential mates as found for large female *Asobara tabida* Nees (Ellers *et al.*, 1998). Larger adults can have higher lipid reservoirs, which can serve as an energy source for dispersal (Rivero and West, 2002). Copulation durations of *C. marginiventris* were several seconds longer in the female choice experiment, where two males were present; however, no male-male fighting was directly observed in *C. marginiventris*. Female preference for larger males appears to occur in the absence of direct male-male competition. Larger males may induce receptivity in females more quickly than small males.

Solitary parasitoids other than *C. marginiventris* have been shown to have a large-male advantage, or exhibit some level of male-male competition. Eggleton (1990) found that large males of the solitary *Lytarmes maculipennis* (Kamath & Gupta) (Ichneumonidae) appeared to mate more frequently than small males. In addition, alternative mating tactics exist for solitary parasitoids. In *Cotesia rubecula* (Marshall), a second male can arrive and steal a mating opportunity from the first courting male (Field and Keller, 1993b).

The female choice experiment for the gregarious parasitoid, *C. flavipes*, suggested that females had no preference for male mate size (Figs. 4.4a, 4.4b), while the male choice experiment suggested that larger males mated with smaller females (Figs. 4.5a-c). Clutches from mated *C. flavipes* typically consist of 80% females (Wiedenmann *et al.*, 1992), so males could be the limited resource. This may preclude females from being selective about mate size, as nearly all attempted copulations by males resulted in matings (Fig. 4.2c).

Male choice experiments showed male *C. flavipes* attempted copulation and mated more frequently with small females than large females (Figs. 4.5a, 4.5b). Small *C. flavipes* females were less likely than large females to reject males on the first attempted copulation (Fig. 4.2d). Although the *C. flavipes* male choice experiment suggested that males choose smaller females, it appears that females ultimately accept or reject the male. During courtship, receptive females stop walking, lower the abdomen and elevate their wings, while unreceptive females jump away and fan their wings. Small females may more readily perceive large males as acceptable mates and signal receptivity; which may explain why males attempted copulations with them first. The larger size difference between males and females in the male choice versus the female choice experiment, may have allowed females to choose larger mates (0.15 mm vs. 0.03 mm, Figs. 4.4c, 4.5c).

Females of *C. glomerata* (L.) (Braconidae), a gregarious parasitoid with a female-biased sex ratio, showed no mating preference between siblings and non-siblings, although the trend was to mate with non-siblings (Gu and Dorn, 2003). In *C. glomerata*, 30% of males and 50% of females dispersed before mating, and some male fighting for females was observed in natal patches where there were large numbers of males. In another study of *Cotesia glomerata*, male size did not influence mating success (Tagawa, 2002). Ode *et al.* (1995) found that females of the gregarious parasitoid

Bracon hebetor Say (Braconidae) were more likely to mate with non-siblings than siblings, possibly because inbreeding produces diploid males with reduced viability. *Melittobia australica* Girault (Eulophidae) has brachypterous males that mate at the emergence site. These males were equally combative with siblings as well as non-siblings (Abe *et al.*, 2005). In *Metaphycus helvolus* (Compere) (Encyrtidae), a solitary parasitoid that attacks clumped hosts, large males approached females more frequently than small males, pushed away small males, and had more attempted and successful copulations than small males (Lampson *et al.*, 1996). In contrast, several other studies of gregarious parasitoids did not suggest a large-male precedence or advantage in mating (Suzuki and Hiehata, 1985; Cheng *et al.*, 2003).

It is not known how female parasitoids might assess male size. Parasitoids are known to perceive color and movement (van den Assem, 1986; Fischer *et al.*, 2004). However, it is unlikely that females of either species considered in this study assess male size visually because males approach females from behind (A.J., unpubl. data). Parasitoid females may use pheromones to asses male size, as in female *Nicrophorus orbicollis* Say beetles (Beeler *et al.*, 2002), although in parasitoids, male pheromones may be less common than female pheromones (Consoli *et al.*, 2002). Chemical cues may have been used when female parasitoids distinguished between siblings and non-siblings (Ode *et al.*, 1995).

A conspicuous aspect of male courtship in *Cotesia* species is their wing fanning behavior, which produces low amplitude sound and substrate vibrations. Female parasitoids have been shown to detect vibrations when searching for hosts (Tomov *et al.*, 2003; Djemai *et al.*, 2004), so may possibly detect substrate vibrations associated with wing fanning. In a study by van den Assem and Putters (1980), older male parasitoids prevented from wing fanning were less successful in mating than young males prevented from wing fanning. However, if courtship vibrations were replayed, mating success improved for the older males. Males of the wolf spider *Hygrolycosa rubrofasciata* (Ohlert) drum their abdomens on leaves to attract females. Females choose males that drum loudest, and this is correlated with male viability rather than male size (Kotiaho *et al.*, 1996; Mappes *et al.*, 1996). Larger male *C. marginiventris* males may produce louder courtship vibrations than smaller males, and induce receptivity in the female more quickly than smaller males.

There may be a large-male advantage to finding females in the field in the solitary species *C. marginiventris*, or competition for mates could happen at the female emergence site, as seen with other solitary parasitoid species (Eggleton, 1990). Evidence of direct competition was not detected in the laboratory, but the mating searching could be examined in a flight chamber to determine if there is a large-male advantage to finding females. Less male competition was observed than expected in the gregarious species, *C. flavipes.* However, gregarious parasitoids may not always exhibit male competition for mates at the emergence site as it may be moderated by dispersal and outbreeding.

CHAPTER V
CONCLUSION

Courtship acoustics and mating behavior were investigated in *Cotesia flavipes*, *C. sesamiae* and *C. marginiventris*. Both airborne and substrate vibrations were recorded simultaneously, and recordings had similar durations and frequencies. Courtship acoustics for each species, and for two strains of *C. sesamiae* were unique. Given that the courtship acoustics were distinct for each species or strain investigated here, the study of parasitoid courtship acoustics has potential to help discriminate members in a species complex, and complement existing techniques, such as molecular and morphological methods of species identification. The role of the mating substrate (plastic, glass, corn leaves, bean leaves, and chiffon fabric) was investigated for *C. marginventris*, and was found to influence the mating frequency and the transmission of courtship vibrations. Chiffon material transmitted courtship vibrations better than other artificial substrates, such as plastic and glass. Additionally, the relative importance of airborne or substrate vibration was investigated, and substrate vibrations were shown to be used for courtship communication in *C. marginiventris.* The importance of the mating substrate and transmission of courtship vibrations may have been overlooked for parasitoids and other insects that use courtship vibrations to communicate. Finally, female and male choice were investigated with *C. marginiventris* and *C. flavipes*. Female choice of larger males was observed in *C. marginiventris*, though male choice of female size was not evident. For *C. flavipes*, female mate choice did not appear to occur during mating, but males mated more frequently with smaller females. Rearing conditions, including the mating substrate, could influence mate choice.

Understanding the role of courtship acoustics for parasitoids can improve our use of these insects for biological control of insect pests. New methodologies to identify species are continuously investigated, and using courtship acoustics to distinguish species or strains could be a complement to existing technologies. Parasitoids detect substrate vibrations, and possibly near-field airborne vibrations (sound) as well. These finding should be further explored with respect to rearing parasitoids and other insects, and can be incorporated into rearing practices in a low-cost manner by selecting cage construction materials that best transmit courtship vibrations. The use of courtship vibrations may be widespread in Braconidae, and investigation of their use in other parasitoid families utilized for biological control deserves attention. The results of the first two studies complemented those of Chapter III, which focused on mate choice. *Cotesia marginiventris* produces substrate vibrations that travel differentially though rearing substrates. *C. marginiventris* females mated preferentially with large males, and visual, chemical or acoustic components of courtship could play roles in mate choice. If vibrational signals are assessed in mate choice, rearing will be impacted the physical properties of the substrate and how they transmit courtship vibrations. Overall, continued research on the role of courtship vibrational communication for these economically important parasitoids warrants further investigation.

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