Complex Vibratory Signalling and Putative Receptor Mechanisms in the Masked Birch Caterpillar, *Drepana arcuata* (Lepidoptera, Drepanidae)

By

Christian Nathan

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Carleton University
Ottawa, Ontario

Abstract

The masked birch caterpillar, *Drepana arcuata*, uses 3 distinct signals when defending its territory from conspecific intruders. The 3 signals are anal scrape, mandible drum and mandible scrape. This study's goals were twofold: first, to test hypotheses on the functional significance of these complex signals, and second, to identify putative vibration receptors in the proleg. Based on experimental trials of size asymmetry certain signal characteristics of the mandible drum and anal scrape were observed to vary between individuals of different mass suggesting the 3 signals could be a result of content based selection and that size information is conferred during an interaction. Trials where the measuring distance was varied, only 2 characteristics of the anal scrape differed significantly between the four recording distances. A dissection study of the proleg discovered that both internal and external structures were innervated. Innervated setae and putative chordotonal organs may function as a multi-component receptor.

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CHAPTER 1

ACOUSTIC COMMUNICATION IN IMMATURE INSECTS

1.1 Acoustic Communication in Insects

Acoustic communication is widespread in insects and has been studied extensively in various species by a number of researchers (Kirchner 1997, Gerhardt and Huber 2002, Greenfield 2002). Acoustic signals in insects serve a variety of functions including courtship (e.g. crickets, Kämper and Dambach 1985 and cicadas, Fonseca 2014) and spacing between conspecifics (e.g. bush crickets, Thiele and Bailey 2006, Virant-Doberlet and Cokl 2004). Various insect species use acoustic communication for defense against predators (Masters 1979). Acoustic displays are used by crickets to display aggression (Alexander 1962, Gabriel et al. 2006), and a number of species of ants use acoustic communication for mediating social recruitment (Kirchner 1997). There are a number of channels or types of acoustic signalling that insects use to communicate.

Acoustic communication encompasses a number of types of signalling which includes airborne, waterborne and substrate signalling. Acoustic signals can be described as the use of mechanical disturbances that propagate through air, water or substrate to communicate (Greenfield 2002, Virant-Doberlet and Cokl 2004, Cocroft and Rodriguez 2005). There are two types of airborne sound: near and far field. Near field of the sound refers to the area around a sound source where propagating molecules travel greater distances. An example can be seen in the honey bee waggle dance, the wing vibrations of the bee produce sound pressures that vary greatly near the bee (Michelsen et al. 1987). Follower bees were observed to place their antennae within this region while the "dancing" bee was signalling. Far field of the sound refers to the outer area around a sound source where the distances the propagating molecules travel return to normal (Bradbury and Vehrencamp 2011). Far field communication occurs over long distances, an example is seen in field crickets, *Gryllus* sp., which produce a high frequency call, usually

above 3 kHz, to attract a mate (Robert et al. 1992, Hoy and Robert 1996). Another form of transmission is solid borne vibrations where the signal, a mechanical disturbance that travels through a solid substrate such as the ground or a plant (Greenfield 2002, Virant-Doberlet and Cokl 2004, Cocroft and Rodriguez 2005). Vibratory communication is used for a number of functions in insects which include mating, spacing, territoriality, group cohesion and coordination, predator deterrence and parent recruitment (Virant-Doberlet and Cokl 2004).

There are four main types of insect acoustic receptors: trichoid sensilla, Johnston's organ, subgenual organ and tympanal organ (Yager 1999, Yack 2004). Trichoid sensilla are innervated hair-like cuticular projections that respond to near field sounds and are found in some species of lepidopteran caterpillars (Markl and Tautz 1975). Johnston's organ is associated with the antenna and also detects near field sounds (Yack 2004). Johnston's organ has been described in the antennae of a mosquito species, *Aedes aegypti*, as well as a leaf hopper species, *Oncopsis flavicollis*, and in honeybees, *Apis mellifera* (Howse and Claridge 1970, Saeng Boo and Richards 1975, Dreller and Kirchner 1993). The subgenual organ is a vibration receptor and is usually located at the tibia of the legs (Virant-Doberlet and Cokl 2004, Yack 2004). Subgenual organs have been characterized as vibration receptors in green lacewings, *Chrysoperla carnea*, carpenter ants, *Camponutas ligniperda*, and honey bees, *Apis mellifera carnica* (Menzel and Tautz 1994, Sandeman et al. 1996, Devetak and Amon 1997). Tympanal organs are the most complex of insect auditory receptors and detect far field sounds (Yack 2004). Tympanal organs have been characterized in a number of insect species across a range of orders including Coleoptera, Dictyoptera, Diptera, Hemiptera, Lepidoptera, Neuroptera and Orthoptera (Yager 1999 and Yack 2004).

The vast majority of studies on insect communication are on airborne communication in adults. However, there is a growing body of literature suggesting that vibratory communication is far more widespread than previously reported, and that immature insects are using acoustic communication. My thesis focuses on vibratory communication and possible reception in immature insects with specific focus on the territorial caterpillar, the masked birch caterpillar *Drepana arcuata*. In this introduction I will review the literature on acoustic communication in juvenile/immature insects focusing on vibratory communication in the larvae and nymphs of holometabolous and hemimetabolous insects. Chapter 2 will focus on the complex vibratory signals of *D. arcuata* to better understand the function of such signals. Chapter 3 will review proposed vibration receptors in immature insects and discuss possible vibration receptors of *D. arcuata.*

1.2 Acoustic Communication in Immature Insects

Despite most of the literature focusing on adults, there is increasing research demonstrating that immature insects also are using acoustic signals for communication. Acoustic signals in immature insects generally serve the same general functions as in adults. With the exception of mating, immature insects use acoustic signals for territoriality, group cohesion, predator deterrence, promoting mutualism and parent recruitment (e.g. Ishay et al. 1974, Devries 1991, Fletcher 2007, Bowen et al. 2008, Low 2008). Acoustic communication channel types include near field, far field and vibration (Cocroft and Rodriguez 2005). Immature insects have not been observed producing near field signals, which involve increasing the movement of molecules around a sound source, however some species of caterpillar possess structures that respond to the near field vibrations of parasitic wasps and flies (Markl and Tautz 1975, Taylor 2008). The use of far field communication has been observed in caterpillars belonging to the

superfamily Bombycoidea for predator deterrence (Bura et al. 2008, 2010, 2012). Vibration communication involves transmitting signals through a substrate either liquid or more commonly solid (Greenfield 2002, Virant-Doberlet and Cokl 2004, Cocroft and Rodriguez 2005). There are a number of examples of immature insects belonging to the orders Coleoptera, Hemiptera, Hymenoptera, Lepidoptera that use vibration communication for various functions (Ishay et al. 1974, Cocroft 2001, Yack et al. 2001, Bowen et al. 2008, Kocarek 2009, Scott and Yack 2012). Acoustic communication in immature insects is a broad topic. This chapter will first discuss communication within the two major divisions of immature insects, hemimetabolous and holometabolous, and then will discuss the functions of acoustic communication within particular taxa of the holometabolous insects.

Most insects hatch from eggs and their development occurs through a series of molts. There are two forms of metamorphosis- holometabolous/complete metamorphosis and hemimetabolous/incomplete metamorphosis. Holometabolous metamorphosis consists of four stages: egg, larva, pupa and adult. Each of the four stages have different morphology. Hemimetabolous metamorphosis consists of three stages: egg, nymph and adult. Unlike holometabolous metamorphosis the nymphal and adult stages of hemimetabolous metamorphosis cannot be distinguished from one another (Gullan and Cranston 2005). Both holo and hemimetabolous immatures have been reported to use acoustic communication.

Communication in immature hemimetabolous insects has not been documented extensively but there are a few examples of vibration communication in treehoppers (Cocroft 2001, *Calloconophora pinguis*, 2005, *Umbonia crassicornis*, Cocroft 1999, Ramaswamy and Cocroft 2009, Hamel and Cocroft 2012). In many treehopper species nymphs are gregarious, living and feeding on the same host plant (Cocroft 2001). Both nymphs and adults communicate

through substrate borne signals (Cocroft 2001, 2005). While the functions of adult treehopper signalling varies the only function described in nymphs is parent recruitment (Cocroft 2001, Ramaswamy and Cocroft 2009). When disturbed the nymphs stop feeding and signal together, when a single nymph is disturbed it will signal first and then it will be followed by the rest thereby providing information on the predator's location (Cocroft 2001). Following the vibration signal of the nymphs the adult female will approach the predator and chase it off and mediate nymph signalling (Hamel and Cocroft 2012). There may be other examples of communication in hemimetabolous insect, however further research is required.

Acoustic and vibration communication has been documented in a number of larvae of holometabolous insects species of Hymenoptera, Coleoptera, Diptera and Lepidotera. The use of near field signals for communication has not been documented in holometabolous insect larvae however a species of caterpillar has been described possessing a receptor which responds to near field acoustic cues generated by predators. Cabbage Moth caterpillars, *Barathra brassicae*, possess 8 filiform hairs each innervated by a single receptor cell located on the dorsal surface of the thoracic segment which function as near field receptors. Caterpillars exhibited defensive responses when these hairs were stimulated, their specific function is thought to detect the wing beat frequencies of tachinid flies or ichneumonid wasps (Markl and Tautz 1975). Markl and Tautz confirmed the role of the thoracic filiform hairs by demonstrating that caterpillars with intact hairs produced defensive responses to predatory wasps. The use of far field signals has been documented in species of Bombycoidea caterpillars which use airborne signals for defense against predators. Sound production has been reported in larvae of the European Great Peacock Moth, *Saturnia pyri*, the North American Walnut Sphinx Moth, *Amorpha junglandis* and the Carolina Sphinx moth, *Manduca sexta* (Bura et al. 2008, 2010, 2012). Both *Saturnia pyri* and

Manduca sexta produce sounds by clicking their mandibles together whereas *Amorpha juglandis* produces sounds by forcing air through an enlarged spiracle located on the eight abdominal segment (Bura et al. 2008, 2010, 2012). In addition to acoustic communication there is an increasing number of examples of immature holometabolous insects that communicate through solid borne vibrations.

There is an increasing number of examples of holometabolous insect larvae that rely on vibration communication (Table 1.1). The functions of vibration communication are extremely varied; solid borne vibrations are used for mediating territoriality (Bowen et al. 2008, Fletcher et al. 2006, Kocarek 2009), interactions with adults (Ishay et al. 1974), mutualistic interactions with heterospecifics (Devries 1990, Travassos and Pierce 2000). Vibrations are also used for detecting predators and parasitoids (Casas et al. 1998, Castellanos and Barbosa 2006, Low 2008). The remainder of this review will provide examples of vibration communication in different orders of larval holometabolous insects.

1.2.1 Hymenoptera

In the order Hymenoptera larval vibration communication has been documented in two species. Ishay (1974) described vibration signals produced by the larvae of the Oriental Wasp, *Vespa Orientalis*. The 3rd, 4th and 5th instar larvae were observed to produce vibrations by bodily contractions within their respective combs; during body contractions the mandibles scrape against the wall of the comb (Ishay et al. 1974). Following food deprivation the larvae were observed to signal more frequently suggesting it may be a hunger signal which was later confirmed through playback experiments where the workers would frequent areas where the vibrator was placed and offer food (Ishay et al. 1974). Playback of the signals at night was

observed to rouse the colony suggesting that the larvae play an important role in the mediation of colony activities (Ishay et al. 1974). Another hymenopterid that uses vibration signals for communication is the Spitfire sawfly larvae, *Perga affinis*; this species lives gregariously in their immature form, feeding in the same area and moving as a unit (Fletcher 2007). Larvae were observed to produce two signals: a tapping and a contraction signal; tapping was produced by striking the substrate with a sclerotized portion of the abdomen while contraction was produced through a fast body twitch (Fletcher 2007). These signals were determined to coordinate group actions such as feeding or movement, tapping occurred while a larva or larvae were feeding on the same leaf or in the same vicinity while contraction signals preceded group movement (Fletcher 2007). Tapping was also observed to play a role in group cohesion; lone larva tapping would elicit a group tapping response from individuals in the vicinity (Fletcher 2007). Stridulation behaviour has been observed in striped alder sawfly larvae, *Hemichroa crocea*, which is proposed to indicate the quality of a food source (Hoegraefe 1984).

Table 1.1 Summary table of current knowledge on vibration communication in immature insects

Insect taxa	Vibration signal	Structure	Proposed Function	Reference
COLEOPTERA Cerambycidae, *Icosium tomentosum*, *Monochamus alternatus*, *Monochamus sutor*	Scratch	Scratches with mandibles	Spacing signal	Kocarek 2009, Izumi et al. 1990, Victorsson and Wikars 1996
Chrysomelidae, *Polychalma multicava*	Tapping, vibrations	Unknown	Aggregation	Windsor 1987
LEPIDOPTERA Drepanidae, *Drepana arcuata*	Drumming, scraping	Drumming with mandibles, scraping with abdomen and mandibles	Territoriality	Yack et al. 2001
Drepana bilineata	Drumming, scraping	Drumming with mandibles, scraping with abdomen	Territoriality	Bowen et al. 2008
Tethea or	Scraping	Scraping with mandibles	Territoriality	Scott and Yack 2012
Gracillariidae, *Caloptilia serotinella*	Scraping, plucking, vibrating	Scraping with mandibles, pulling with mandibles and thoracic legs, vibration of thorax	Territoriality	Fletcher et al. 2006

Table 1.1 Summary table of current knowledge on vibration communication in immature insects

Heliozelidae, *Antispila nysaefoliella*	Tick and rattle	Tick produced with lateral movement of abdomen, rattle produced by vibrating abdomen	Predator deterrence	Low 2008
Lycaenidae, *Jalmenus evagoras*	Drumming, stridulation	Unknown	Ant mutualism	Travassos and Pierce 2000
Riodinidae, *Thisbe irenea*	Stridulation	Vibratory papillae	Ant mutualism	Devries 1990
Eurybia elvina	Scraping	Epicranial granulations	Ant mutualism	Travassos et al. 2008
HEMIPTERA Membracidae, *Calloconophora pinguis*, *Umbonia crassicornis*	Vibrating	Whole body vibration	Attract adults for defense	Cocroft 2005, Cocroft 1999, Ramaswamy and Cocroft 2009, Hamel and Cocroft 2012
HETEROPTERA Tingidae, *Corythucha hewitti*	Vibration	Abdomen	Aggregation	Cocroft 2001
HYMENOPTERA Tenthredinoidea, *Perga affinis*	Tapping and contraction	Tapping with abdomen and contraction with whole body	Group coordination	Fletcher 2007
Hemichroa crocea	Stridulate and scratching	Stridulate with body and scratching with abdomen	Group coordination and food quality	Hoegraefe 1984
Vespidae, *Vespa orientalis*	Scraping	Scraping with mandibles	Attract adults for feeding	Ishay et al. 1974

1.2.2 Coleoptera

The use of vibration communication has been documented in a few larval coleopterans across two main families (Cerambycidae, Izumi et al. 1990, Victorsson and Wikars 1996, Kočárek 2009 and Scarabaeidae, Kojima et al. 2012). The use of vibrations by larvae of the non-laminae longicorn, *Icosium tomentosum*, is proposed to be used to space themselves within a host tree (Kočárek 2009). Signal production is thought to occur when the larvae scratch against the galleries with their sclerotized mandibles (Kočárek 2009). Chorusing behaviour was observed in this species as well; when one larva signals this causes others to signal which Kočárek believes to reduce localization by predators and parasitoids. Similar signalling behaviour is seen in the species *Monochamus alternus* and *Monochamus sutor* (Izumi et al. 1990, Victorsson and Wikars 1996). In all three species, vibration communication is thought to function in spacing, these larvae are highly territorial and will fight and cannibalize each other if their galleries come in contact (Izumi et al. 1990, Victorsson and Wikars 1996, Kocarek 2009). Recent studies conducted by Kojima et al. (2012) found that pupae of the Japanese rhinoceros beetle, *Trypoxylus dichotoma*, produce vibrations. The pupae and larvae of these species inhabit the same area; burrowing larvae can damage the delicate pupae; the pupae produce a vibration signal that causes a freezing response in the larvae (Kojima et al. 2012). The signal is produced when the protonum is struck against the inner pupal cell wall (Kojima et al. 2012). The freezing behaviour is thought to be an anti predator response which is exploited by the pupae (Kojima et al. 2012).

1.2.3 Diptera

Vibration signals have been documented as being a part of hunting behaviour in larval Diptera specifically in the family Culicidae (Jackson 1953 and McIver and Beech 1985). Two species of mosquito larvae which have been observed to respond to vibrations (*Culex tigripes*, Jackson 1953 and *Toxorhynchites brevipalpis*, McIver and Beech 1985). Both species were observed to use vibrations for prey detection (Jackson 1953, McIver and Beech 1985). *Culex tigripes* larvae were observed to move when the container holding them was vibrated, Jackson (1953) postulated that movement following vibration stimulation may be related to the predaceous habit of the larvae. *Toxorhynchites brevipalpis* larvae attacked a glass probe when vibrated at particular frequencies suggesting the larvae are sensitive to vibrations that are produced by moving prey and use these vibrations for detection (McIver and Beech 1985).

1.2.4 Lepidoptera

A number of species of larval Lepidoptera use vibration signals for the purposes of communication; the function of the signals includes promoting mutualism with heterospecifics, deterring predators and mediating territoriality (Devries 1991, Travassos and Pierce 1999, Yack et al. 2001, Fletcher et al. 2006, Bowen et al. 2008 and Travassos et al. 2008). These signals can range from being simple to complex. Examples for each function are discussed below.

Larval Lepidoptera are a common food source for predators and parasitoids. Some species communicate with ants to promote mutualism (*Thisbe irenea*, Devries 1991, *Jalmenus evagoras*, Travassos and Pierce 1999 and *Eurybia elvina*, Travassos et al. 2008). Devries reports that *T. irenea*, a riodinid, is not the only riodinid to produce calls; Devries (1991) states that 19 species of riodinid and 30 species of lycaenid displayed the ability to call. The riodinid species

tested possess two structures, vibratory papillae and epicranial granulations, that varied in morphology (*Synargis gela*, *Juditha molpe*, *Menander menander*, *Calospila cilissa*, *Calospila emylius*, *Theope thestias*, *Theope virgilius* and *Nymphidium mantus*, Devries 1991). Interestingly the lycaenids tested possessed epicranial granulations but lacked vibratory papillae; their exact method of vibration production has yet to be discerned (Devries 1991). The vibratory papillae are ringed peg like structures located at the back of the head, these are rubbed against epicranial granulations which are hardened studs located at the top of the head directly below the papillae (Devries 1991). The caterpillars produce their calls by oscillating their head so that the papillae grate against the granulations; differences in the calls were observed between riodinids and lycaenids (Devries 1991). Calls differed in terms of notes, pulse rates and dominant frequency as well as the frequency range; the distance these signals can travel however is unknown (Devries 1991). Larval and pupal calls have been observed in the Australian common imperial blue butterfly, *Jalmenus evagoras*, which are attended by the ant species *Iridomyrmex anceps* (Travassos and Pierce 1999). The larval signals consist of a grunt, hiss and drum; these signals differ in terms of their characteristics and serve to attract ants to attend to them (Travassos and Pierce 1999). Travassos et al. (2008) studied *Eurybia elvina*, a riodinid, that lacked vibratory papillae but still was capable of attracting ants; the signal was produced by grating the epicranial granulations against the prothorax. It was noted that ants concentrated around the area where the signal was being produced (Travassos et al. 2008).

A second function for vibratory signalling in Lepidoptera larvae is defense against predators. There is only one species of Lepidoptera larvae that use vibration signals for deterring predators. The larvae of the Tupelo leafminer, *Antispila nysaefoliella*, emit vibratory signals when disturbed which may function to deter predators (Low 2008). There are two signals

produced by the larvae: the tick and rattle. The tick is longer in duration and is produced by scraping the abdomen back and forth across the leaf surface (Low 2008). The rattle signal is produced by vibrating the abdomen very rapidly (Low 2008). The signals are produced by sclerotized structures on the dorsal side of the abdominal and caudal segments and caudal tip (Low 2008).The function of these signals has not been determined but since these larvae are parasitized the signals thought to disrupt foraging parasitic wasps (Low 2008).

The use of vibrations for territoriality has been observed in larval Lepidoptera of differing families. The cherry leaf roller, *Caloptilia serotinella*, which belongs to the family Gracillariidae produces territorial vibrations in response to a conspecific. The cherry leaf roller was observed to produce three signals: scraping, plucking and vibrating (Fletcher et al. 2006). These signals were produced in response to a conspecific intruder entering a resident's leaf shelter (Fletcher et al. 2006). Scraping is produced by a lateral head movements while the mandibles are held open and scraped against the leaf surface (Fletcher et al. 2006). Vertical movement of the head and thorax produce the plucking signal; either the mandibles or the thorax pulling up on the leaf are thought to produce the signal (Fletcher et al. 2006). The vibrating is produced when the larva vibrates horizontally against the leaf surface, the thoracic legs scraping the leaf are thought to produce the signal (Fletcher et al. 2006). The signals differed in usage when interacting with a conspecific; the most frequently used signal is scraping followed by plucking and vibrating (Fletcher et al. 2006). The distance between the resident and the intruder also determined which signal was used, at farther distance vibrating was observed while at closer distances scraping was observed (Fletcher et al. 2006). A number of larvae of the family Drepanidae produce territorial vibrations specifically in the subfamilies Drepaninae and Thyatirinae (Scott et al. 2010). Some examples include the warty birch caterpillar, *Drepana*

bilinetea and the masked birch caterpillar, *Drepana arcuata* (Yack et al. 2001, Bowen et al. 2008, Scott et al. 2010). Both species have multiple signals which escalate over the course of an interaction (Yack et al. 2001, Bowen et al. 2008). The warty birch caterpillar, *Drepana bilineata*, defends an occupied leaf from conspecifics using two signals: mandible drumming and anal scraping (Bowen et al. 2008). The mandible drum signal is produced when the caterpillar raises its head and strikes the leaf with its mandibles while the anal scrape is produced when the caterpillar moves its abdomen in an anterior direction while a modified setae scrapes against the leaf surface (Bowen et al. 2008). Signalling rates increased as intruders got closer to residents with mandible drums and anal scrapes being used with equal frequency during signalling bouts (Bowen et al. 2008).

Another drepanid that uses mandible drums and anal scrapes is the masked birch caterpillar, *Drepana arcuata*, however this species has a third signal included in its signal repertoire, the mandible scrape (Yack et al. 2001) (Fig. 1.1). This species differs from *D. bilineata* in terms of its life history, *D. arcuata* lives gregariously in its first two instars and constructs a full silk shelter while *D. bilineata* lives solitarily and only constructs a silk mat in its later instar (Yack et al. 2001). Residents were observed to increase the rate of signalling and varied the use of particular signals in relation to distance between the intruder; at closer distances the use of the mandible scrape signal increased significantly (Yack et al. 2001). Signalling rates increased with the amount of time a caterpillar spent on a leaf as well as with silk accumulation (Yack et al. 2014). Conversely signal rates decreased with shelter removal (Yack et al. 2014). Although the signals have shown considerable variation a resounding question remains, why do these caterpillars require three distinct signals for communication?

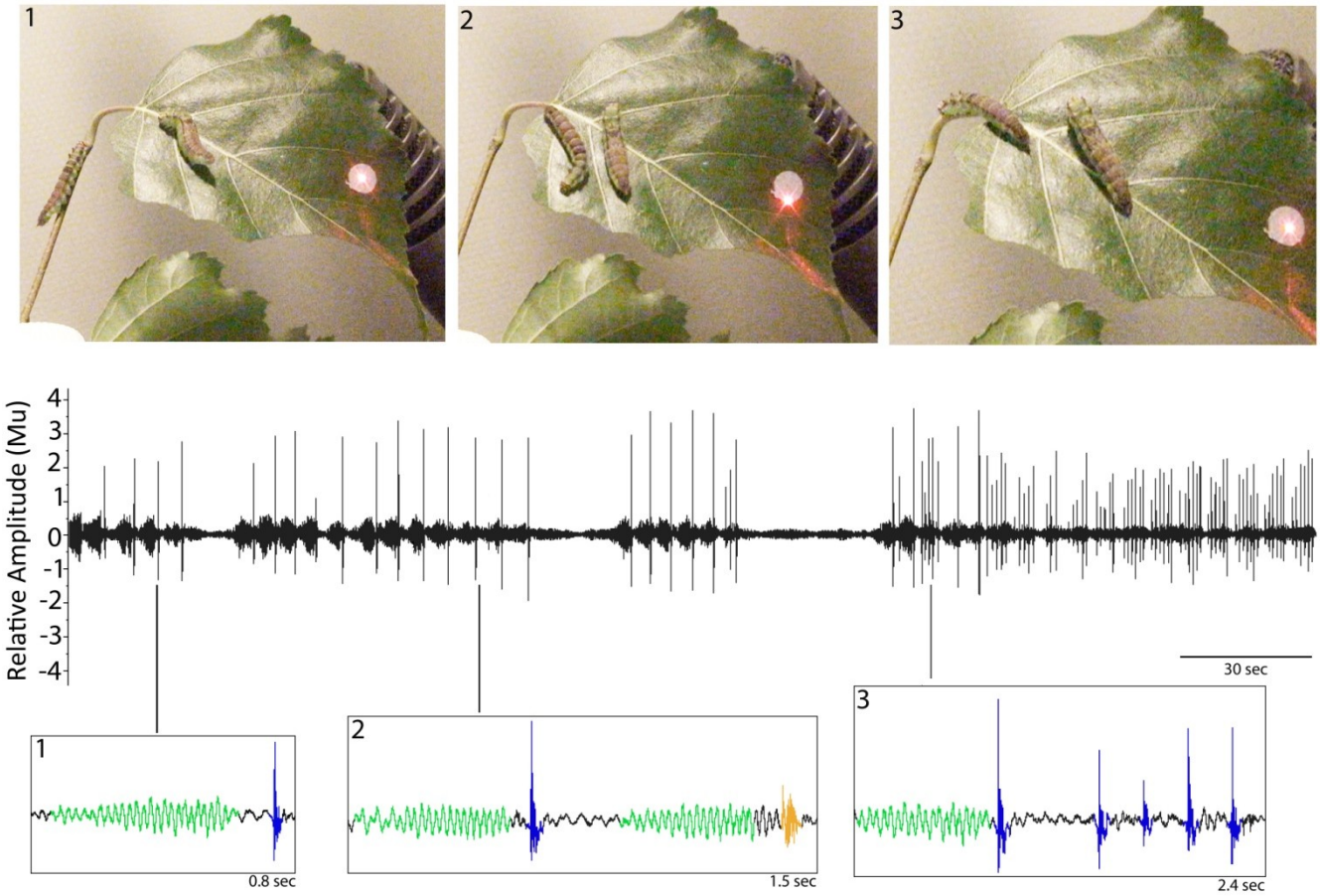

Figure 1.1 Photo of *D. arcuata* territorial interaction and waveform of signals produced during the interaction recorded by laser vibrometer. Photos and waveforms are numbered according to the stage of the interaction. The signals are colour labelled, anal scrapes are green, mandible drums are blue and mandible scrapes are orange. (1 photo) Early stage where the intruder is ascending the petiole of the leaf. (1 waveform) The resident produces anal scrapes and mandible drums only. (2 photo) Intruder has entered the resident's territory. (2 waveform) The resident has now incorporated mandible scrapes into its display. (3 photo) Intruder leaves territory after extended signalling by the resident. (3 waveform) As the intruder leaves the territory the resident uses fewer mandible scrapes but continues to display until the intruder has left the leaf.

1.3 Vibratory Receptor mechanisms in Immature Holometabolous Insects

Despite the growing evidence that holometabolous insect larvae use vibrations for communication and that some of these vibrations are complex and directed at conspecifics, there is virtually no direct evidence for vibration receptors in larval holometabolous insects. The list of possible vibration receptors includes pleural discs, setae, setae and apodemum and antennae. All the aforementioned structures are putative vibration receptors, none have had their proposed function confirmed. Pleural discs were described in Cerambycid larvae, they are multi-component structures that are thought to be arranged to detect sounds of differing characteristics (Hess 1917). Setae in two species of Lepidoptera have been described being innervated by a sense cell, these setae may function to detect the near field vibrations of wasps and parasitoids (Markl and Tautz 1975, Castellanos et al. 2011). In the superfamilies Pyraloidea and Gelechioidea, larvae possess an apodemum connected to elongated setae which may function in detecting vibrations transmitted through a silk shelter (Hasenfuss 1992). The antennae are multi-component structures that may play a role in vibration detection due to the sensilla that comprise it (Dethier 1941, Meurgey and Faucheux 2006). Further discussion of putative vibration receptors in immature insects in general, and in *D. arcuata* are found in Chapter 3.

Thesis objectives

The main objective of my thesis is to better understand vibration reception in the masked birch caterpillar, *D. arcuata*. This will expand the existing knowledge base on vibration communication in holometabolous insect larvae as it is the first time a vibration receptor is characterized in the larvae of a holometabolous insect species that use substrate-borne vibrations for the purposes of communicating. My thesis will involve studying the territorial signals of *D.*

arcuata as well as identifying and describing the components of a receptor in an effort to understand vibration reception in this species. There are 2 main goals of this study, the first goal covered in chapter 2 is to record and characterize the territorial signals of *D. arcuata* to determine why this species uses 3 distinct signals and what the signals mean. This will provide information on what characteristics a potential receptor may be sensitive to in order to distinguish between the 3 signal types. The second goal is covered in chapter 3, characterizing potential receptors of *D.arcuata* through morphological studies. This will provide information on the location and the components that comprise a vibration receptor in this species. Characterization of the receptors will offer insight on how the receptor's components function to receive a signal.

Chapter 2

Signal characteristics and their function in the territorial displays of the masked birch caterpillar, *Drepana arcuata* (Drepanoidea: Drepanidae)*

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Introduction

Research on animal communication signals focuses on discerning their function, design and evolution (Hebets and Papaj 2005). These signals can be complex and are organized into three categories: multicomponent, multimodal or variation within a component (Hebets and Papaj 2005). Multicomponent signalling contains multiple components or displays, which elicit a behavioural response in the receiver- however the individual components will not (Hebets and Papaj 2005). Multicomponent signals occur in one sensory modality (Rowe 1999). Nestling cuckoos produce a multi-component call composed of a rapid succession of individual 'si' calls to simulate a brood of chicks to increase the provisioning rate of adults (Davies et al. 1998). Multimodal signals refer to signals that are composed of more than one signal in more than one sensory modality (Hebets and Papaj 2005). Mating displays of several species of spider are examples of multimodal signals where males produce a vibratory signal through tapping their abdomen and forelegs and a visual signal through their tufts of hair on their forelegs or by raising their forelegs (Hebets and Uetz 1998, Girard et al. 2011, Wilgers and Hebets 2011). The vibration signals are used for long distance attraction but also play a role in female choice while the visual signals are when the female can view the male, both signal types play a role in female receptivity. However, isolated vibration and visual signals will still elicit responses in the female if the species uses multimodal signalling (Hebets and Uetz 1998). Variation within a component refers to changes to a signal characteristic (Guilford and Dawkins 1993, Hebets and Papaj 2005). Songbirds have been shown to be able to control the amplitude of their songs when competing against rival males or adjust amplitude with distance from a receiver (Brumm and Todt 2003, Brumm and Slater 2005).

Explanations for why signals vary have been reviewed by a number of authors, and hypotheses explaining variation can be categorized based on content, efficacy and inter-signal interaction (Guilford and Dawkins 1993, Hebets and Papaj 2005). This study focuses on two of these hypotheses- content and efficacy. Content based selection states that the function of a signal relates to the message it is trying to convey through a complex display (Hebets and Papaj 2005). The American goldfinch, *Carduelis tristis*, uses ornamental traits to convey different information, the black cap of the bird provides information about social interactions while the plumage and bill coloration are indicators of intestinal infection (McGraw and Hill 2000). Efficacy based selection states that the function of a signal relates to the efficacy with which it traverses an environment and is processed by a receiver (Hebets and Papaj 2005). Tawny owls increase their calling range 70-fold by calling during dry conditions as opposed to rainy conditions (Legange and Slater 2002). This paper seeks to understand why complex signalling occurs in a territorial caterpillar, *Drepana arcuata*, that communicates with conspecifics using vibratory signals.

The masked birch caterpillar, *D. arcuata*, produces complex signal patterns comprised of 3 different signal types during territorial encounters with conspecifics, but the reason for using 3 distinct components during these interactions is not clear. The components that comprise the acoustic display are an anal scrape, mandible drum and mandible scrape. The anal scrape is produced when the caterpillar drags a pair of chitinous structures known as anal oars located on the abdomen in the anterior direction, the mandible scrape is produced when the caterpillar strikes the leaf surface with its open mandibles while the mandible scrape is produced when the caterpillar moves its head in a lateral direction while holding open the mandibles against the leaf surface. The masked birch caterpillar is common throughout northeastern North America; early

instars are gregarious. When the caterpillars reach the third instar they build solitary silken shelters on leaves (Rose and Lindquist 1997, Yack et al. 2001). The late instar caterpillars that first arrive on the leaf and construct the silk shelters are referred to as the residents whereas conspecifics that arrive afterwards are known as the intruders. As an intruder walks down the petiole into the resident's territory, the resident stops its activity and backs into its shelter and begins signalling (Yack et al. 2001). When a conspecific intruder comes on the leaf, the resident begins a complex signal display that can include 2 to 3 of the components. The display usually comprises anal scrapes and mandible drums earlier on during an interaction however as the interaction progresses and the intruder gets closer mandible scrapes are incorporated into the display (Fig. 1.1). In addition, each of these signal types been reported to vary in rate as the intruder encroaches on a signaler's territory. During territorial interactions there is variation in both signal type and rate (Yack et al. 2001). Although we know there is variation within an interaction, the reason for there being 3 signals is still unknown.

Research to date has focused primarily on how the rates of these signals change over the course of an encounter, how the rates differ between contestants, and proximate factors that affect signalling (Yack et al. 2001, Guedes et al. 2012, Yack et al. 2014). As stated previously, the display initially consists of anal scrapes and mandible drums, however, as the display progresses mandible scrapes are included in the display. During an interaction residents have been observed to vary signal rates during the stages of the encounter, as the intruder approaches closer and enters the shelter. When an intruder is further away from the shelter mandible drums and anal scrapes are used, at mid-distance the mandible drum rate increases significantly and when the intruder is close the mandible scrape rate increases and the 3 signals are used equally.

The change in signal type and rate over the course of an encounter was interpreted as escalation (Yack et al. 2001).

Caterpillars were also observed to vary signal rate in relation to their motivation to defend a territory. Signalling effort was observed to vary depending on the amount of time spent on a leaf, caterpillars that had spent 3 minutes or longer on the leaf established themselves as the residents and signaled more than intruders (Yack et al. 2014). Signalling increased in relation to silk accumulation, when placed on a leaf with fully constructed shelters caterpillars were observed to signal more than caterpillars placed on bare leaves (Yack et al. 2014). Signalling decreased when a resident's silk shelter was removed from the leaf (Yack et al. 2014). Despite our knowledge on the varying signal rates there is little we know about how the 3 signal types differ physically. We also do not know why there would be a need for 3 different signal types since escalation of an encounter and motivation levels could be conveyed by varying one signal type. Thus, determining why these caterpillars use 3 signals is an important question. In this study we pose 2 general hypotheses that are not necessarily mutually exclusive:

1. The different signals convey different meanings in general (content based hypothesis)

2. The different signals are effective over different distances (efficacy based hypothesis)

Different signals can have different meaning; this falls under the category of content based selection which states a signal's function relates to its message. The multiple messages hypothesis states that each signal type or characteristic provides different information or information on different aspects of signaler quality (Hebets and Papaj 2005). Male jumping spiders, *Phidippus clarus*, produce 2 distinct vibrations when courting a female, a courtship signal and an aggressive courtship signal. Researchers focused on the courtship signal and found

that signal rate was positively correlated to size (Sivalinghem 2010). In *D. arcuata*, residents usually win territorial interactions, but when an encounter is staged between small resident and large intruder as determined by mass, the intruder takes over. Intruders that were significantly heavier than the residents won interactions (Yack et al. 2001). Additionally experiments on residency duration and territory quality discerned that caterpillars signal more when they have spent more time on the leaf or have a fully constructed shelter (Yack et al. 2014). Each signal could provide different information the previous studies have indicated that size and motivation are pieces of information that could be transmitted. I have outlined the content based hypotheses I will be testing below.

Experiment 1 Hypothesis (content based)

The 3 signals produced by *D. arcuata* have multiple meaning

Experiment 1 Prediction:

There are differences between the signals within a given individual in terms of their characteristics. The 3 signals, anal scrape, mandible drum and mandible scrape will vary in terms of the measured signal characteristics.

Experiment 2 Hypothesis (content based)

One or more signals provide information about size

Experiment 2 Prediction:

Between the 3 signals, 1 or more signals provides more information about size. The mandible drum signal is produced by lifting the head and thorax off the leaf and striking it causing a displacement, thus a heavier caterpillar will cause a larger displacement. I predict as caterpillars of higher mass are compared to caterpillars of lower mass the peak amplitude of the mandible drum signal will be observed to increase. I predict the anal scrape duration will increase in larger caterpillars since they are longer in body length.

Some animals' signals are multicomponent because they are better at being transmitted in different conditions or may travel further than other signals, this is a hypothesis that falls under the category of efficacy based selection. Efficacy based selection states the function of a signal relates to the efficiency with which it travels through an environment (Hebets and Papaj 2005). The wolf spider, *Schizocosa retrorsa*, uses both visual and vibratory displays in courtship and was observed to obtain an equal amount of copulations in both dark and light conditions and on substrates that either transmitted vibrations or did not suggesting that the types of signals used varied with the environment (Hebets et al. 1996, Hebets and Uetz 1999). In *D. arcuata* signals were observed to vary in rate at different distances perhaps this is due to some signals being more efficient in travelling certain distances through the environment. The 3 signals may also be effective at different distances. I have outlined the efficacy based hypothesis I will be testing below.

Experiment 3 Hypothesis (efficacy based)

There are 3 signals because each signal is more effective at different distances.

Experiment 3 Prediction:

The characteristics of the 3 signals produced by *D.arcuata* will vary at different distances from the source. I predict specifically that signal spectral and amplitude features will vary between the 3 signals at different distances.

Methods

Insects

Adult *D. arcuata* females were field-collected from various sites near Ottawa, Ontario, Canada. The eggs were laid and larvae reared on cuttings of paper birch (*Betula papyfera*). All larvae used in experiments were those occupying solitary shelters, in their third, fourth and fifth larval instars. The larvae were reared under normal light levels (18:6) and room temperature (21-26^{o}C). All experiments were conducted between the months of June and October, 2012 and 2013. 10 individuals were used in experiment 1. 19 individuals were used in experiment 2. 82 individuals were used in experiment 3.

General Recording Setup

Prior to recording the trials, two caterpillars were selected to act as the resident and intruder. The mass of each caterpillar was obtained using a balance (CP224 S, Sartorius) and was used to define caterpillar size. The resident caterpillar was placed on a birch leaf attached to a 6 to 8 cm long twig placed in a plastic vial filled with water. Leaves were pre-selected to be within 5–7 cm wide and 6–9 cm in length. The resident was left undisturbed for at least 6 hours on the

isolated leaf to allow it to build its shelter. The intruder was isolated in a glass vial with a bare birch twig 15 minutes prior to the experiment. A laser disc was placed 2 cm from the resident prior to the interaction. Recording began as soon as the intruder was deposited 2-3 cm under the petiole of the leaf and was stopped following the intruder leaving the resident's territory or until the necessary signals were collected for the particular experiment (see below). All trials that were videotaped used a Sony video camera (Handycam HDV 1080i/MiniDV, Sony). In some trials a microphone was attached to the camera, the microphone was placed 1-2 cm behind the leaf (Electret condenser microphone, Sony). All trials were carried out inside an acoustic chamber (C-14A MR, Eckel, Morrisburg, ON, Canada).

Vibration recording and signal measurements

Leaf vibrations produced by resident caterpillars and conspecifics were recorded with a laser vibrometer (PVD-100, Polytec Inc., Ann Arbor, MI, USA). The laser output was low-pass filtered at 22 kHz and digitized at 48 kHz and recorded to a data recorder (PMD 671, Marantz, New York City, NY, USA). Temporal and spectral characteristics of the vibrations were analyzed using Raven Pro v. 1.4 (Cornell Laboratory of Ornithology, Ithaca, NY, USA). To assess velocity for a subset of individuals, the voltages of the largest mandible drums within a signalling bout were measured directly from the oscilloscope (TDS-2002, Tektronix, Beaverton, OR, USA) from the analog output of the laser vibrometer.

General Acoustic Sampling

Not all signals were analyzed from each .wav file, but rather, were sampled based on the following general procedures. For each file, I aimed to obtain values for 10 of each signal type. Since mandible scrapes were always fewer in number than the other signals, this was the limiting

factor in selecting files. Mandible drums and anal scrapes were sampled from the 1st drum/scrape and then every 5th drum/scrape. For mandible scrapes if there was a large number produced within a file every 5th was sampled if not every 4th, 3rd and 2nd depending on the number of scrapes in the file. The method of sampling described above was applied to all the experiments.

Signal Analysis

For signal analysis, I used Raven Pro version 1.4 (Cornell Laboratory of Ornithology, Ithaca, NY, USA). A waveform (relative amplitude versus time), spectrogram (frequency versus time) and power spectrum (relative power versus frequency) was generated for all signals sampled (see below for specifics on sampling, Fig. 2.1). The spectrogram was used to determine the onset of a signal, the increase of energy from background level was determined as the start of a signal. Power spectra were produced using an 8192-point Fast Fourier Transform (Hann window, 50% overlap). Background was sampled with the same duration as the signal being measured. The measuring window was kept at the same duration as the signal and moved to an area where no signalling was observed. The background was subtracted from all spectral and temporal characteristics. Duration (s), the total length of the signal, was measured off the waveform (Charif et al. 2010).

To measure the spectral characteristics of the signals maximum power and maximum frequency were recorded off the spectrogram, maximum power (dB relative to 1 dimensionless sample unit) refers to the higher power within the selection denoted by the darkest region of the spectrogram and maximum frequency (Hz) is the frequency at which maximum power occurs within a selection. Bandwidth (Hz) was measured off the power spectrum and refers to the

frequency range of the signal. Bandwidth was measured at -6, -12, -24 and -48 dB to capture a range of frequencies (Charif et al. 2010).

To measure the amplitude characteristics of the signals peak amplitude and RMS amplitude were measured off the waveform. Raven units are relative units that are applied to the signals within a wav. file based on the sampling frequency and settings of the recorder. Peak amplitude (Raven units) is the greater of the absolute values of maximum and minimum amplitude. RMS amplitude (Raven units) is the root-mean-square amplitude which is essentially the average amplitude of the samples within a selection, the size of the measuring window was kept at the same duration of the signal (Charif et al. 2010). The RMS amplitude values were multiplied by duration to compare between the signal types. A direct measure of amplitude was obtained by converting the voltage measurements to velocities using a scaling factor obtained from the laser manual (mm/s). A scaling factor is a value that expresses the conversion rate of volts to mm/s based on the velocity setting of the laser.

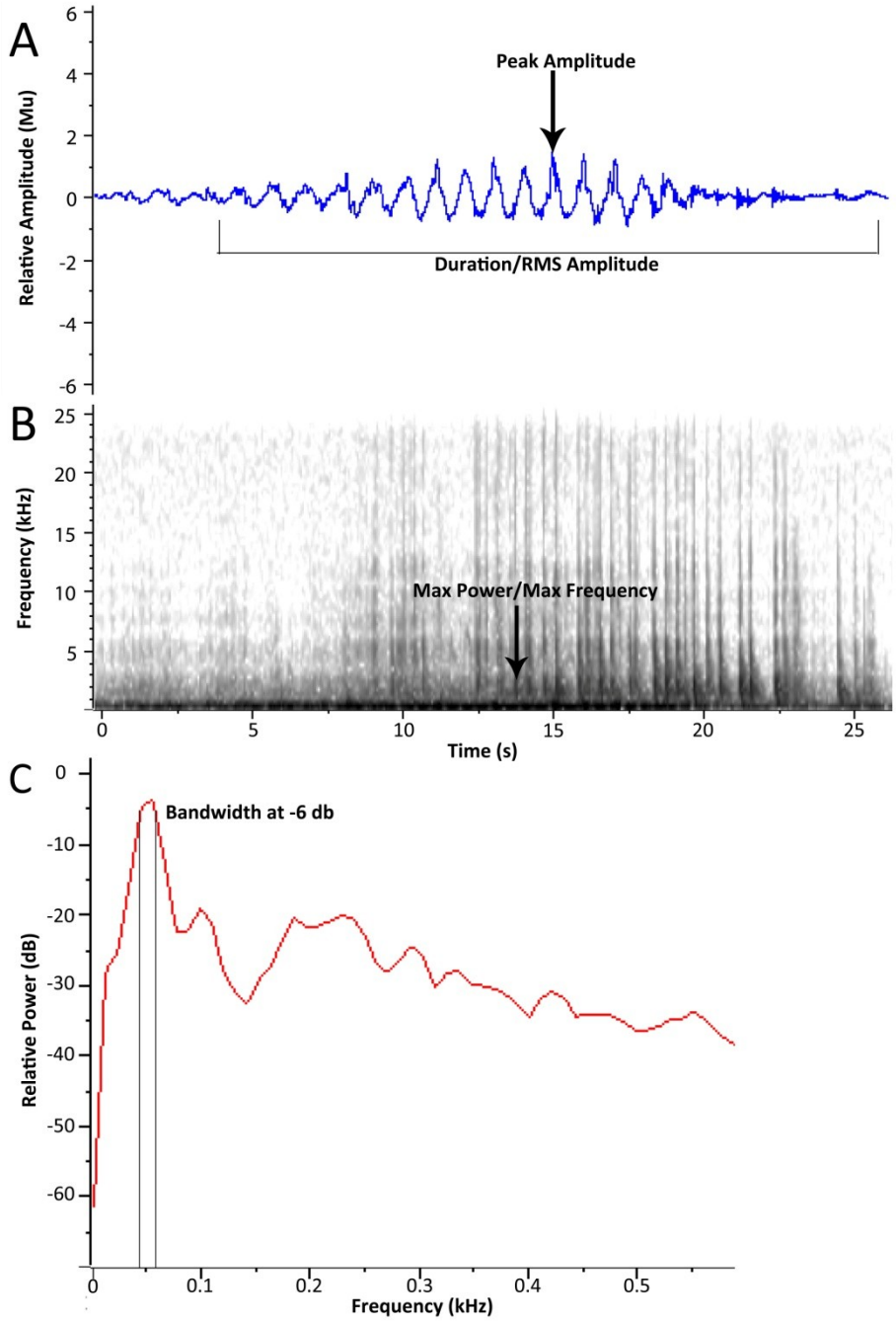

Figure 2.1 Raven measurement windows and measured signal characteristics. (A) Oscillogram of an anal scrape. Signal characteristics measured off the oscillogram include duration, peak amplitude and RMS amplitude. (B) Spectrogram of an anal scrape. Maximum power and maximum frequency are measured off the spectrogram. (C) Power spectrum of an anal scrape. Bandwidth is measured off the power spectrum.

Experiment 1: Do the 3 signals vary between each other within an individual?

The purpose of this experiment was to determine whether each of the 3 signals differed significantly from one another with respect to the measured temporal, spectral and amplitude characteristics described above. For this experiment two sized matched individuals as defined by mass were used, one serving as the resident and one as the intruder. The setup and introduction of the intruder follow the procedure outlined previously. Acoustic and video recording commenced as soon as the intruder was placed on the petiole and was stopped when either the resident produced mandible scrapes or signalled using only anal scrapes and mandible drums for at least 1 minute. The voltage measurements were obtained only after a 10 second period of constant signalling, 3 measurements were taken at approximately 10 second intervals. The distance between the resident and the laser disc was monitored throughout the trial, and the trial would be stopped if the resident moved 2 cm away from the disc as determined by visual observation and measurement using a ruler. The vibration recordings were sampled as described in the general analyses above. Details on statistical analyses are described below.

Experiment 2: Do the 3 signals vary between individuals of differing size?

To assess whether signals vary with the size of the individual, two caterpillars of differing size were used. Mass was used to determine size: caterpillars that were 0.006 to 0.009 g were considered small, caterpillars weighing 0.01 to 0.05 g were considered medium sized while caterpillars weighing 0.06 to 0.1 g were considered large. The intruder size was fixed within the medium mass range, while the resident's size was varied to be either larger or smaller than the

intruder. The setup and introduction of the intruder follows the procedure outlined previously. Different leaves of the same size were used in this experiment since each resident needed to establish a shelter to defend. The interaction was recorded and filmed as described in experiment 1. The vibration recordings were sampled as described in the general analyses above. Three voltage measurements of the largest mandible drums were obtained as the trial progressed. The voltage measurements were obtained only after a 10 second period of constant signalling, 3 measurements were taken at approximately 10 second intervals. Details on statistical analyses are described below.

Experiment 3: How do the 3 signals vary with distance?

The third experiment aimed to determine whether and how the 3 signals varied with distance from the source, across the leaf. The experiment consisted of an interaction between two size-matched individuals as determined by mass. Unlike previous trials the interaction was recorded at 4 different distances, 1, 2, 4 and 6 cm away from the resident. The setup and introduction of the intruder follows the procedure outlined previously with the exception of the laser disc. Four laser discs would be placed at 1, 2, 4 and 6 cm away from the resident. All 4 laser discs were placed on the leaf. The distances were chosen based on observations on the distance between caterpillars and the onset of signalling from previous experiments (Yack et al. 2001, Guedes et al. 2012). The interaction was recorded and filmed as described in experiment 1. The vibration recordings were sampled as described in the general analyses above. Details on statistical analyses are described below. Direct voltage measurements were not assessed in this particular experiment.

Statistical Analyses

The results of **experiment 1** (intra-individual comparisons) were subjected to a repeated measures analysis of variance with signal type acting as the independent variable and the signal characteristics as the dependent variable. A *post-hoc* Bonferroni correction, at $p < 0.05$, was performed on the results of the analysis of variance. For **experiment 2** (interindividual comparisons-size) regression analyses were performed between the resident caterpillar's mass (independent variable) and signal characteristics (dependent variable). In **experiment 3** (distance trials) signalling was measured at 3 different distances, and a repeated measures analysis of variance was performed on the results with signal type at each distance acting as the independent variable and signal characteristics as the dependent variable. The results of the analysis of variance were subjected to a *post-hoc* Bonferroni correction at $p < 0.05$.

Results

Experiment 1: Do the 3 signals vary between each other within an individual?

The masked birch caterpillar produces 3 signals in a territorial display; each is produced by a distinct body movement which suggests the signals may vary in terms of their characteristics. Ten recordings were obtained from 10 different individuals in experiment 1. Differences were observed between the 3 signals in terms of their duration, peak amplitude and RMS amplitude (Fig. 2.2). A significant difference in duration was observed when the 3 signals were compared to one another (ANOVA: $F_{2,18}=38.301$, $p < 0.001$, partial $R^2=0.810$), anal scrapes

were longer in duration (0.624 ± 0.192 s, n=10) than the mandible drum (0.217 ± 0.069 s, n=10) (Bonferroni: p=0.001) and the mandible scrape (0.212 ± 0.088 s, n=10) (Bonferroni: p<0.001) (Fig. 2.3). When peak amplitude was compared between the signals there was a significant difference (ANOVA: $F_{2,18}$=10.919, p = 0.001, partial R^2=0.548); mandible drums were higher in peak amplitude (2791.391 ± 2184.469 kU, n=10) than mandible scrapes (1195.027 ± 1181.841, n=10) (Bonferroni: p=0.012) (Fig. 2.4). The RMS amplitude of the signals showed a significant difference (ANOVA: $F_{2,22}$=10.434, p < 0.001, partial R^2=0.487) with mandible scrape having a lower RMS amplitude (72.8252 ± 72.2033 kU, n=12) than either anal scrapes (171.051 ± 116.607 kU, n=12) (Bonferroni: p=0.008) or mandible drums (117.612 ± 80.3472 kU, n=12) (Bonferroni: p=0.017) (Fig. 2.5).

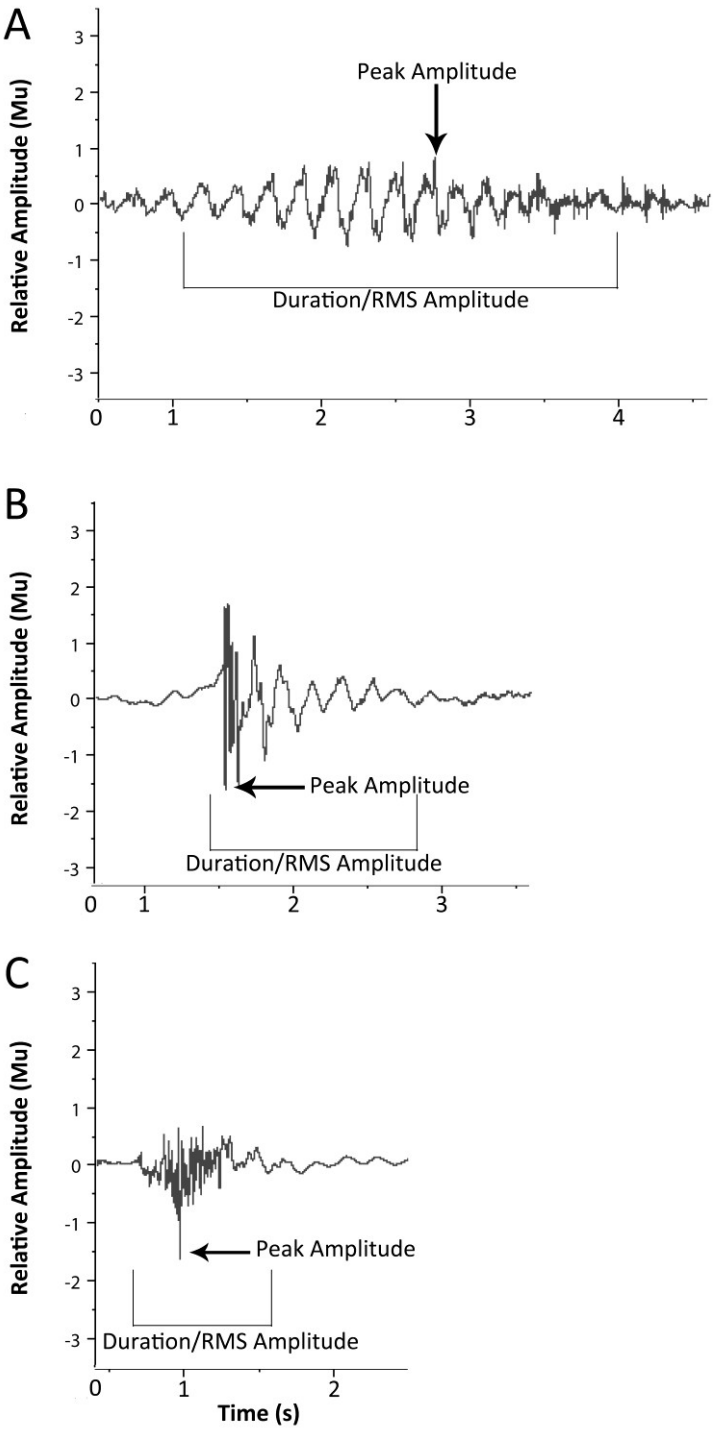

Figure 2.2 Experiment 1: Within individual comparison of the oscillograms of the 3 signal types. (A) Oscillogram of an anal scrape. (B) Oscillogram of a mandible drum. (C) Oscillogram of a mandible scrape. Note the differences in duration, peak amplitude and RMS amplitude.

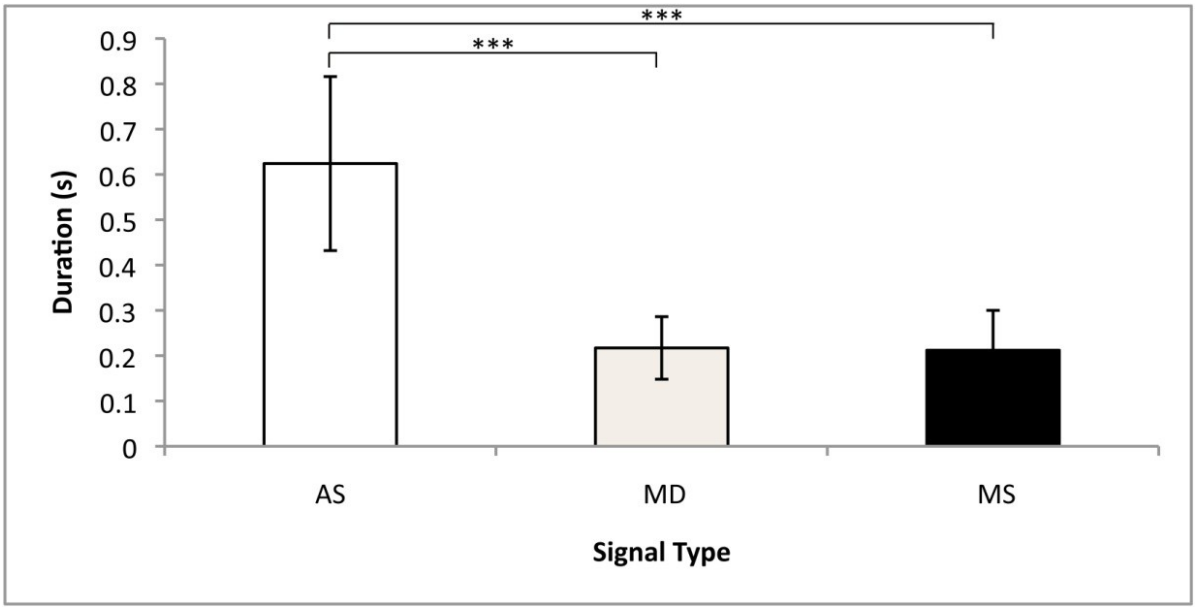

Figure 2.3 Experiment 1: Within individual comparison of mean duration (s) between signal types. Note the significant difference in duration between the anal scrape (AS) and the mandible drum (MD) and mandible scrape (MS).

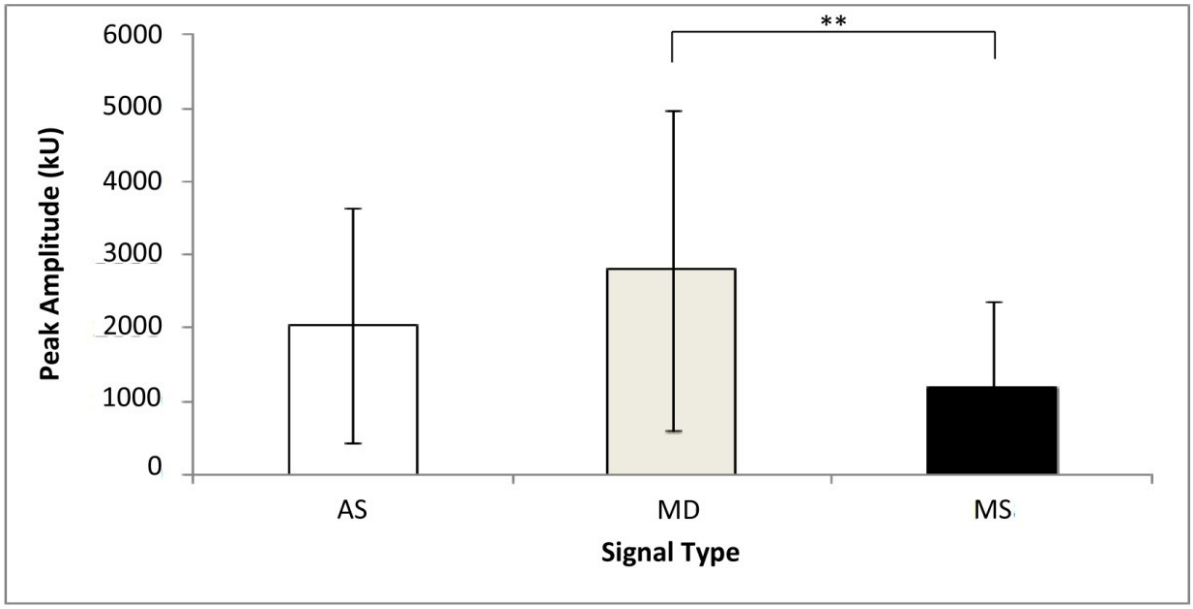

Figure 2.4 Experiment 1: Within individual comparison of mean peak amplitude (kU) between signal types. Note the significant difference between the peak amplitude of mandible drums (MD) and mandible scrapes (MS).

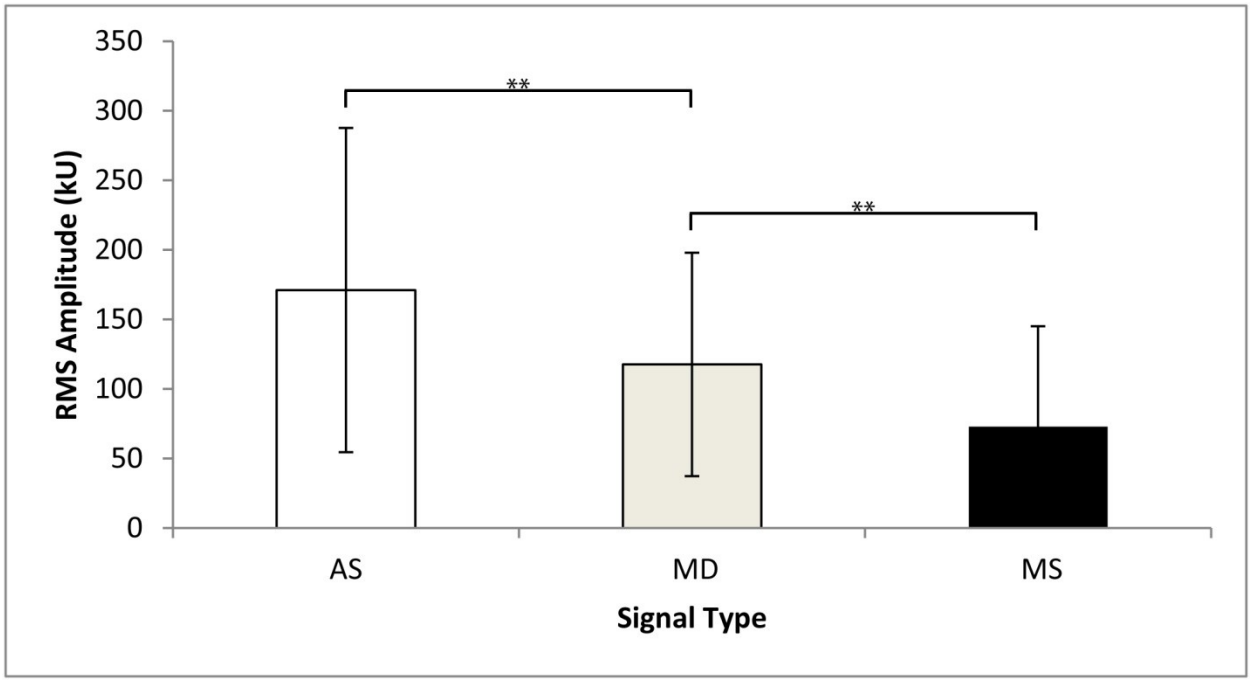

Figure 2.5 Experiment 1: Within individual comparison of mean RMS amplitude (kU) between signal types. Note the significant difference between anal scrapes (AS) and mandible drums (MD) as well as between mandible drums and mandible scrapes (MS).

Experiment 2: Do the 3 signals vary between individuals of differing size?

Nineteen recordings were obtained from 19 individuals of different mass in experiment 2. Voltages were obtained from 96 individuals of different mass. Differences were observed between each of the signal types between individuals of different mass (Fig. 2.6-8). Larger caterpillars produced drums of longer duration (R^2=0.271, p = 0.009) (Fig. 2.9). Larger caterpillars produce drums of higher velocity (R^2=0.389, p < 0.001) (Fig. 2.10). Mandible scrapes also showed variation in peak amplitude between masses in the positive direction (R^2=0.265, p = 0.060) (Fig. 2.11). A significant difference was also observed in mandible drum RMS amplitude which increased when mandible drums were produced by larger caterpillars (R^2=0.251, p = 0.029) (Fig. 2.12). The anal scrape RMS amplitude also increased positively with size (R^2=0.252, p = 0.025) (Fig. 2.13).

Experiment 3: How do the 3 signals vary with distance?

The signal characteristics of the 3 signal types may travel different distances. For experiment 3, 15 recordings were obtained at the 1 cm distance, 10 recordings were obtained at the 2 cm distance, 31 recordings were obtained at the 4 cm distance while 26 recordings were obtained at the 6 cm distance. When comparing characteristics within each signal type measured at the 3 distances only anal scrape duration (ANOVA: $F_{3,12}$=5.111, p = 0.017) and peak amplitude (ANOVA: $F_{3,12}$=8.314, p = 0.003) showed significant difference. The anal scrape was longer in duration than the other 2 signals at all 4 recording distances, the anal scrape duration decreased as the measurement distance increased (Fig. 2.14). The durations of all 3 signals were highest at 2 cm and diminished as the laser disc was placed further away (Fig. 2.14-

2.16). The mean peak amplitude for the anal scrape was higher at 2 cm (2100.165 kU ± 850.984 kU) then it decreased as the recording distance was moved further away (Fig. 2.17). The mandible drum had the highest peak amplitude over all 4 recording distances and increased as the laser disc was moved further away from the resident (at 1 cm 1765.086 kU ± 2293.484 kU, at 4 cm 4185.705 kU ± 2759.479 kU) (Fig. 2.18). When RMS amplitude was compared all 3 signals decreased in amplitude when the 1 cm and 6 cm measurements were compared. The decrease in RMS amplitude was not steady in all 3 signals, either at 2 cm or 4 cm the amplitude increased before decreasing in subsequent distances (Fig. 2.20-22).

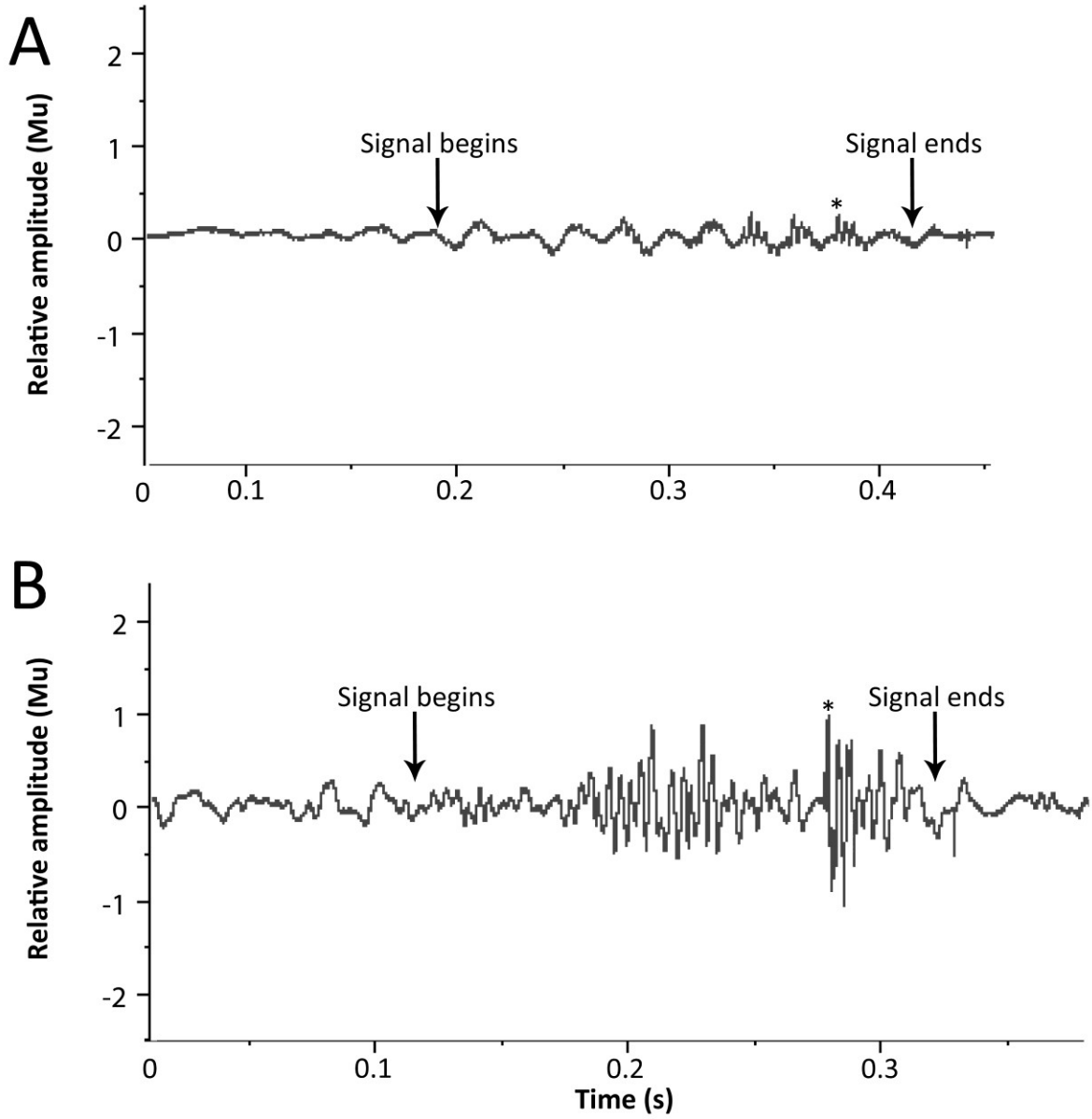

Figure 2.6 Experiment 2: Comparison of anal scrape between small and large resident. (A) Oscillogram of an anal scrape of a small resident, 0.0062 g. (B) Oscillogram of an anal scrape of a large resident, 0.1108. Asterisk marks peak amplitude in both diagrams. Peak amplitude of anal scrape produced by the small resident is 332.3 kU. Peak amplitude of anal scrape produced by the large resident is 821 kU.

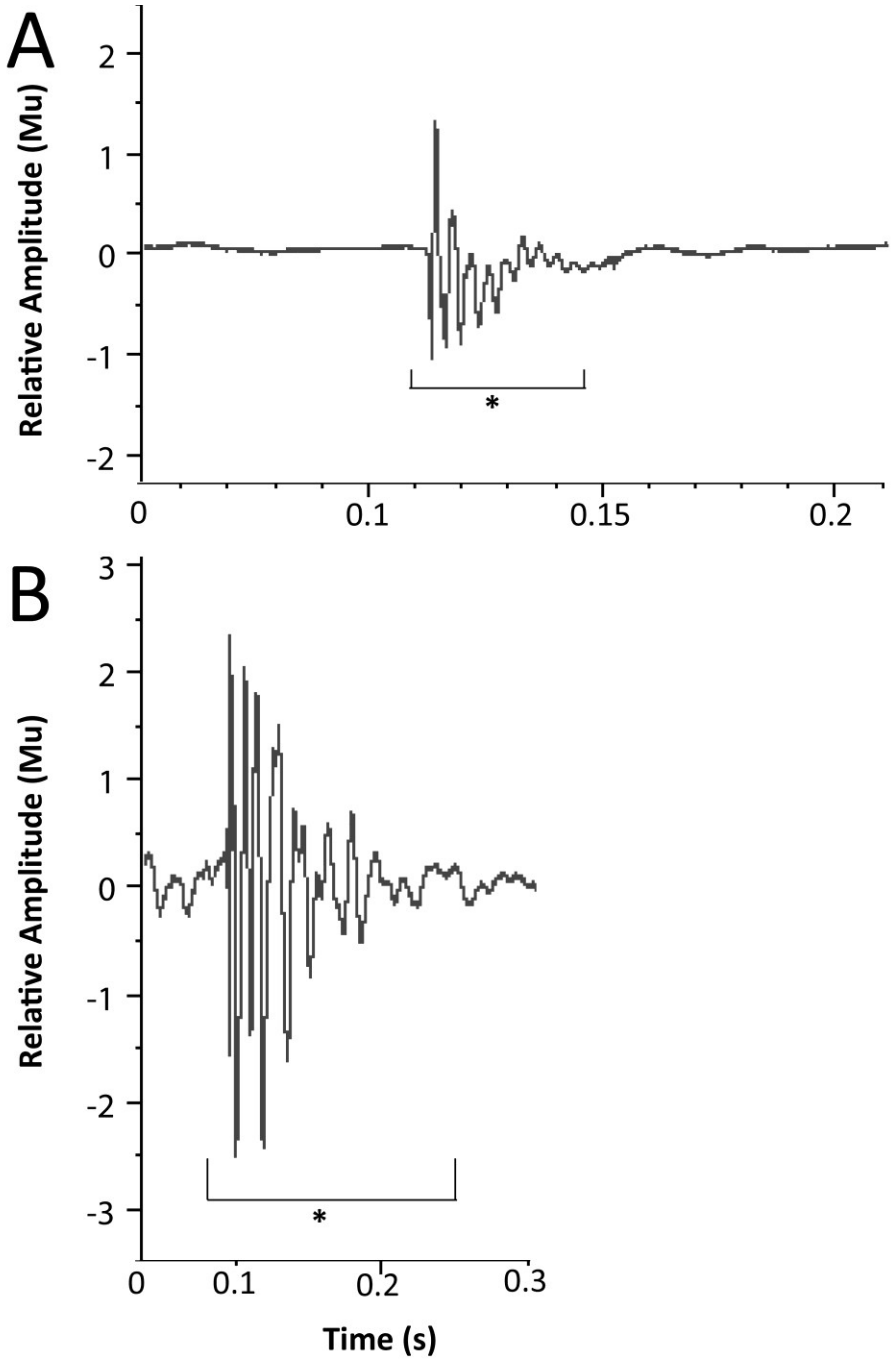

Figure 2.7 Experiment 2: Comparison of mandible drum between small and large resident. (A) Oscillogram of a mandible drum of a small resident, 0.0062 g. (B) Oscillogram of a mandible drum of a large resident, 0.1108 g. Asterisk marks duration in both diagrams. The duration of the mandible drum produced by the small resident is 0.03 s while the duration of the mandible drum produced by the large resident is 0.15 s. Peak amplitude of the mandible drum produced by the small resident is 102 kU while the peak amplitude of the mandible drum produced by the large resident is 376.6 kU.

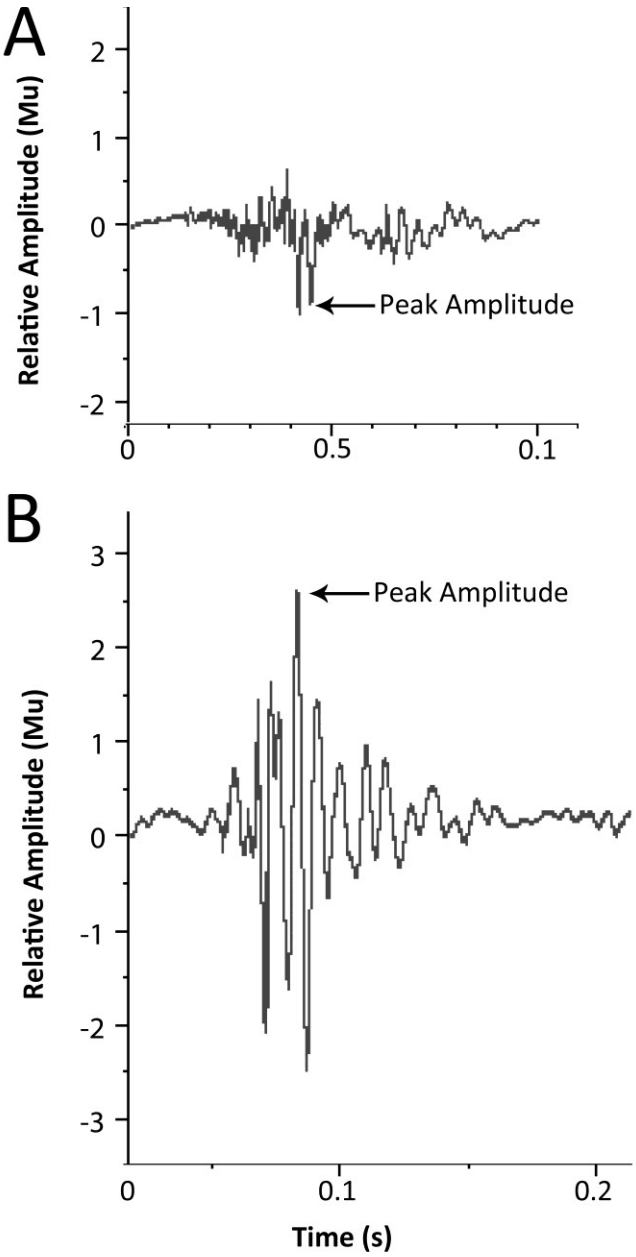

Figure 2.8 Experiment 2: Comparison of mandible scrape between small and large resident. (A) Oscillogram of mandible scrape produced by a small resident, 0.0062 g. (B) Oscillogram produced by a large resident, 0.1108 g. The peak amplitude of the mandible scrape produced by the small resident is 114 kU while the peak amplitude of the mandible scrape produced by the large resident is 905 kU.

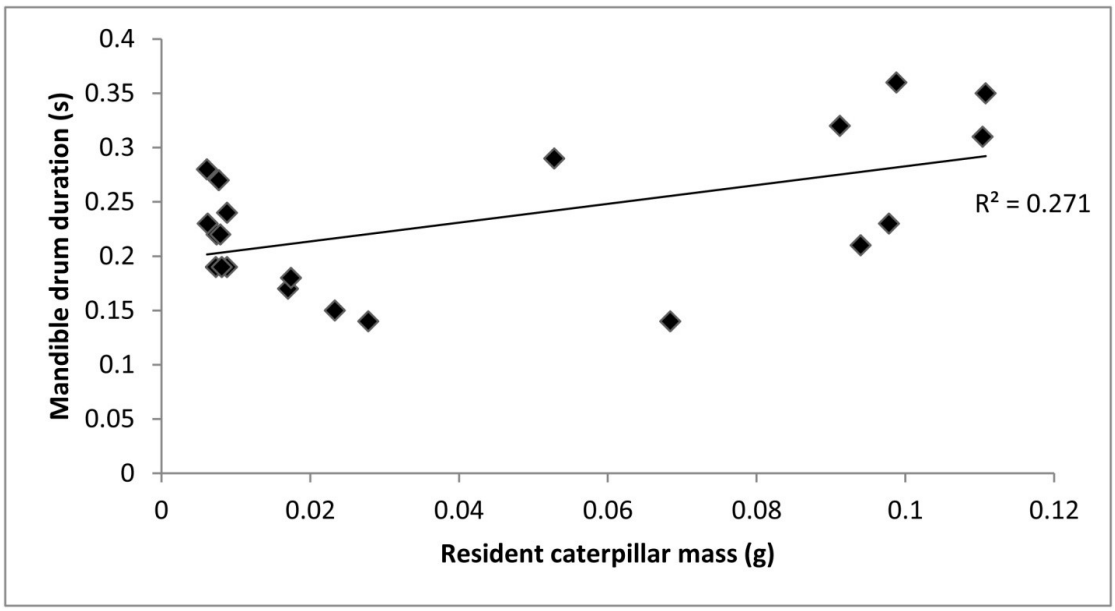

Figure 2.9 Experiment 2: Regression analysis of mandible drum duration (s) and resident caterpillar mass (g).

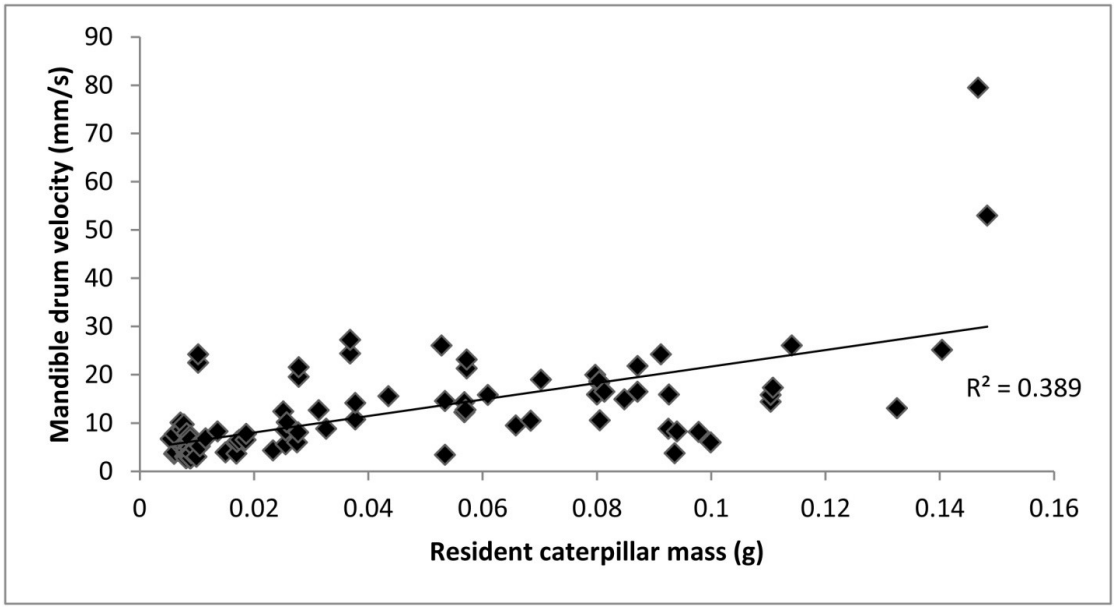

Figure 2.10 Experiment 2: Regression analysis of mandible drum velocity (mm/s) and resident caterpillar mass (g).

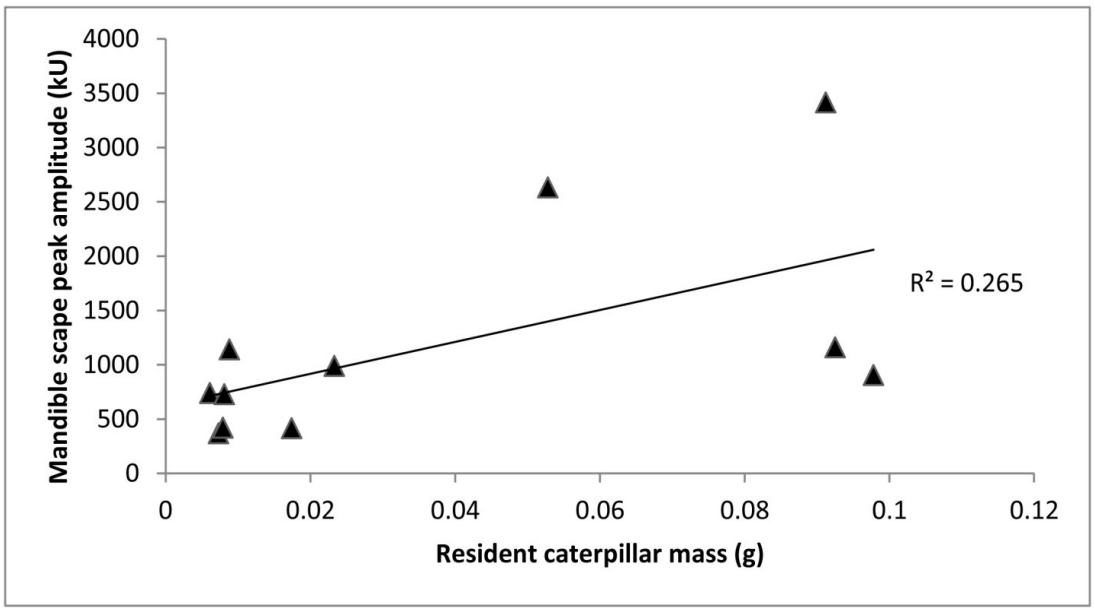

Figure 2.11 Experiment 2: Regression analysis of mandible scrape peak amplitude (kU) and resident caterpillar mass (g).

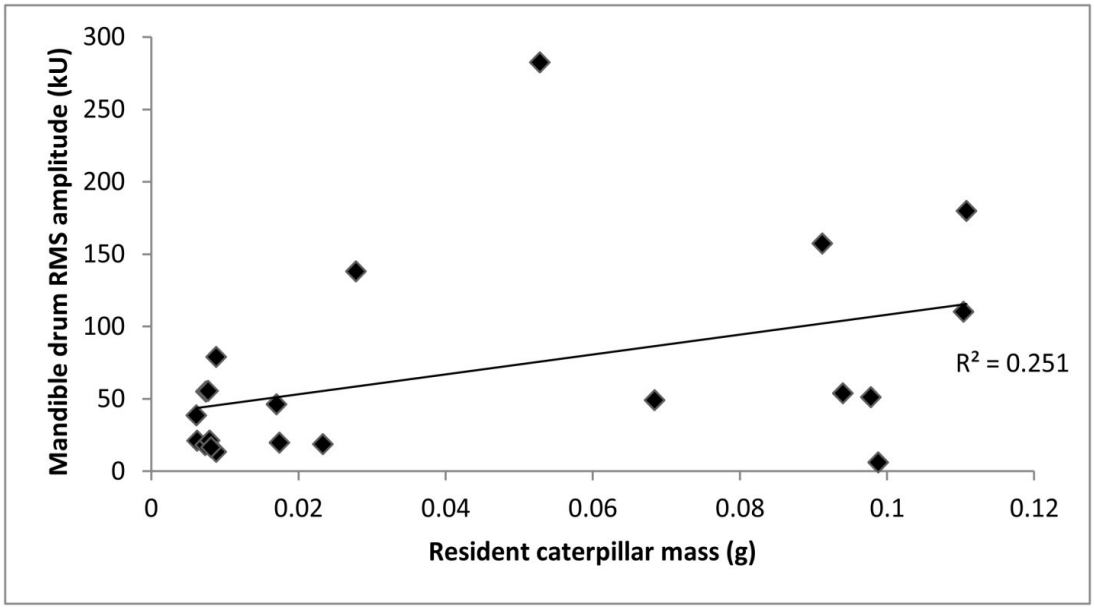

Figure 2.12 Experiment 2: Regression analysis of mandible drum RMS amplitude (kU) and resident caterpillar mass (g).

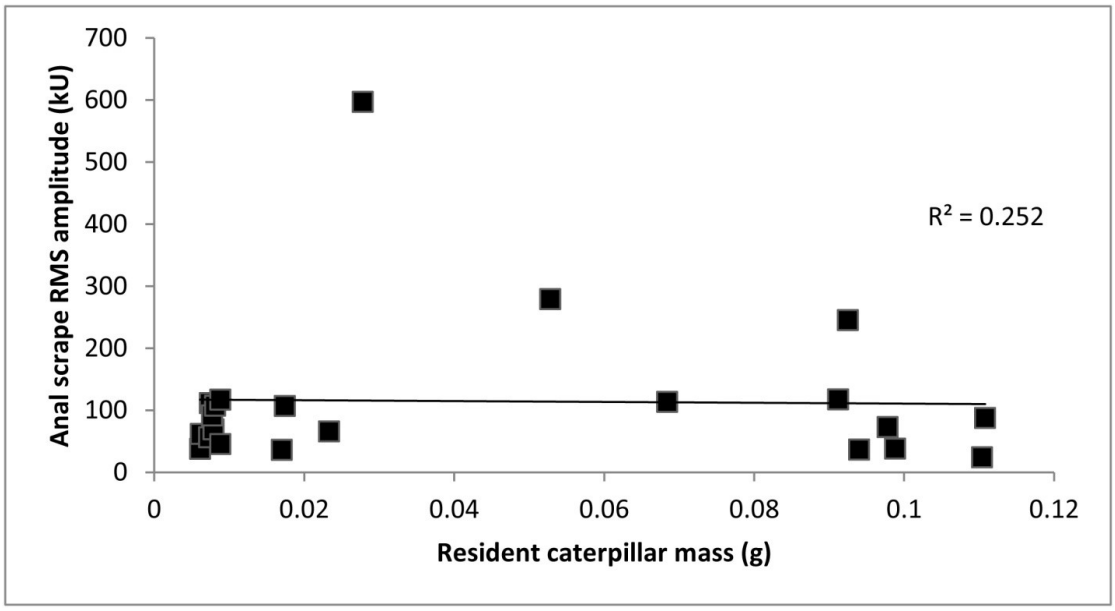

Figure 2.13 Experiment 2: Regression analysis of anal scrape RMS amplitude (kU) and resident caterpillar mass (g).

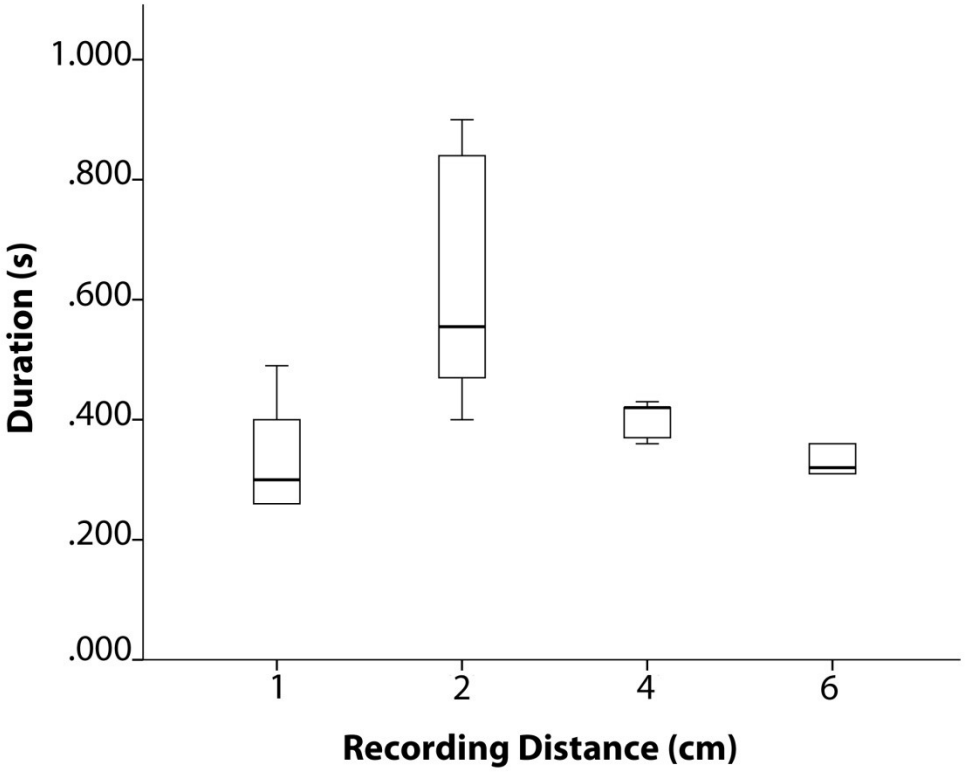

Figure 2.14 Experiment 3: Comparison of anal scrape mean duration (s) between four different recording distances.

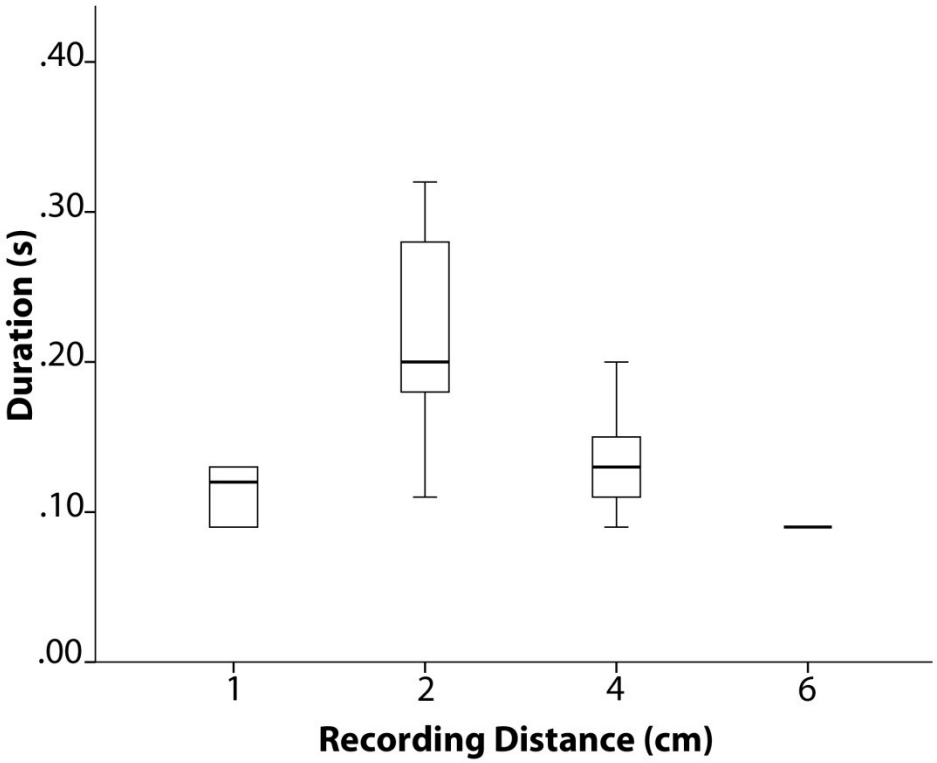

Figure 2.15 Experiment 3: Comparison of mandible drum mean duration (s) between four different recording distances.

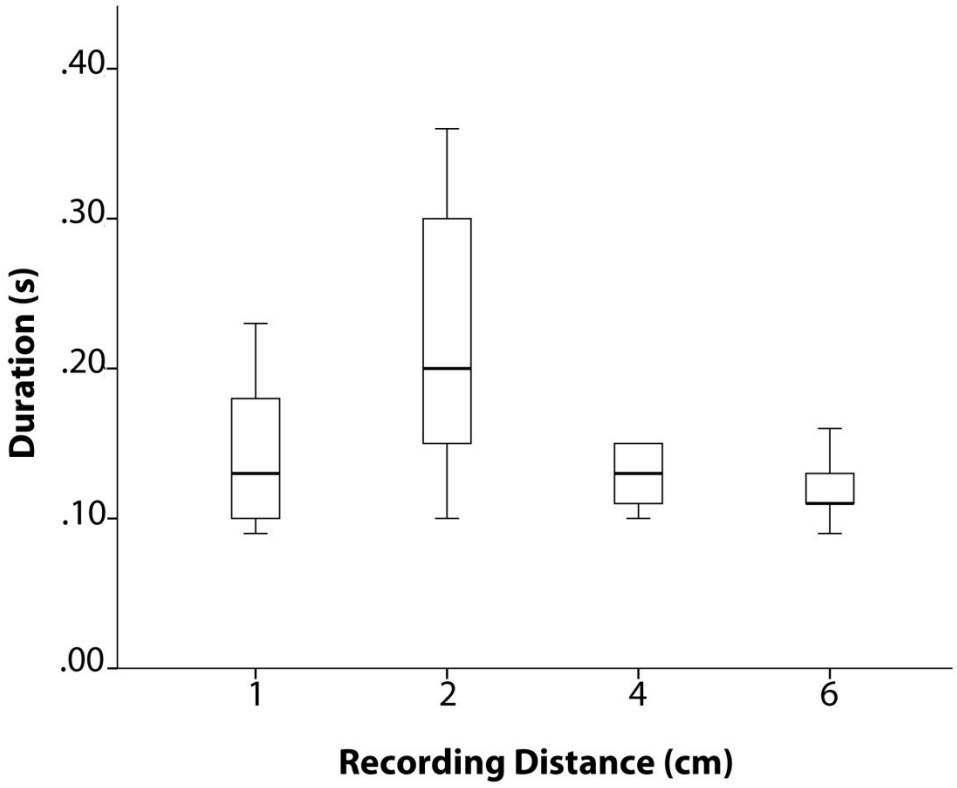

Figure 2.16 Experiment 3: Comparison of mandible scrape mean duration (s) between four different recording distances.

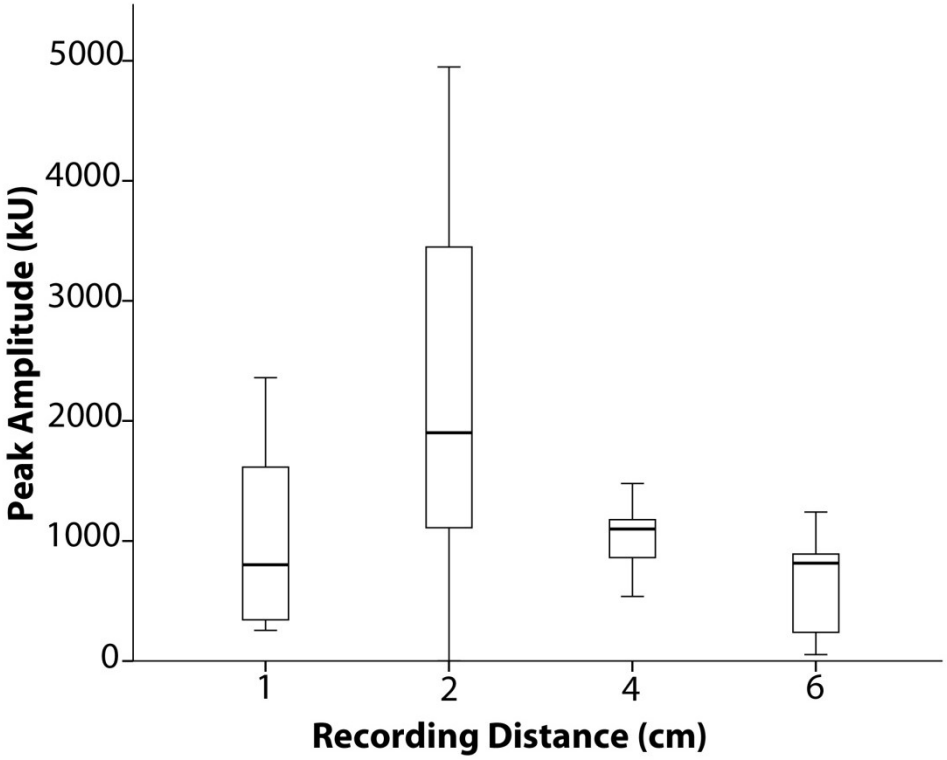

Figure 2.17 Experiment 3: Comparison of anal scrape mean peak amplitude (kU) between four different recording distances.

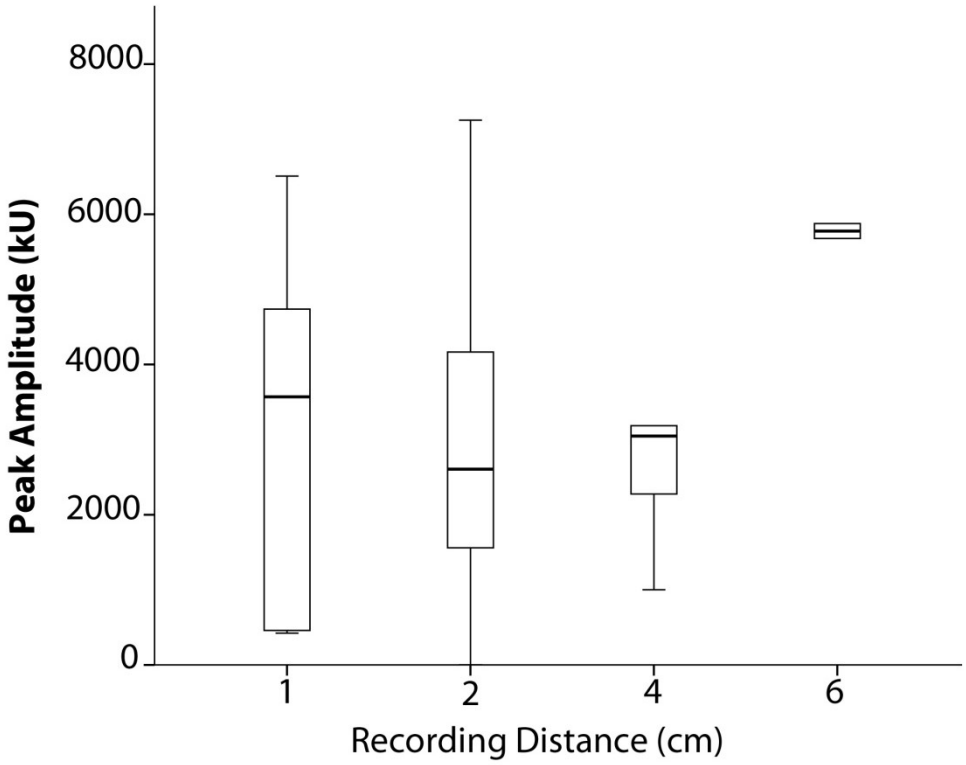

Figure 2.18 Experiment 3: Comparison of mandible drum mean peak amplitude (kU) between four different recording distances.

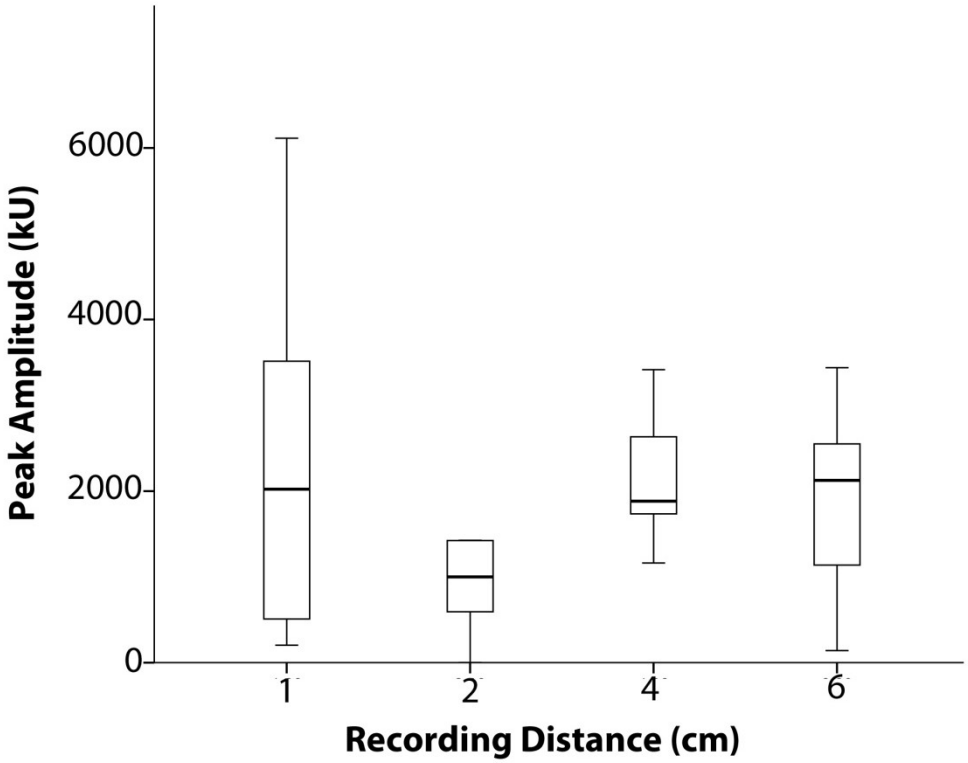

Figure 2.19 Experiment 3: Comparison of mandible scrape mean peak amplitude (kU) between four different recording distances.

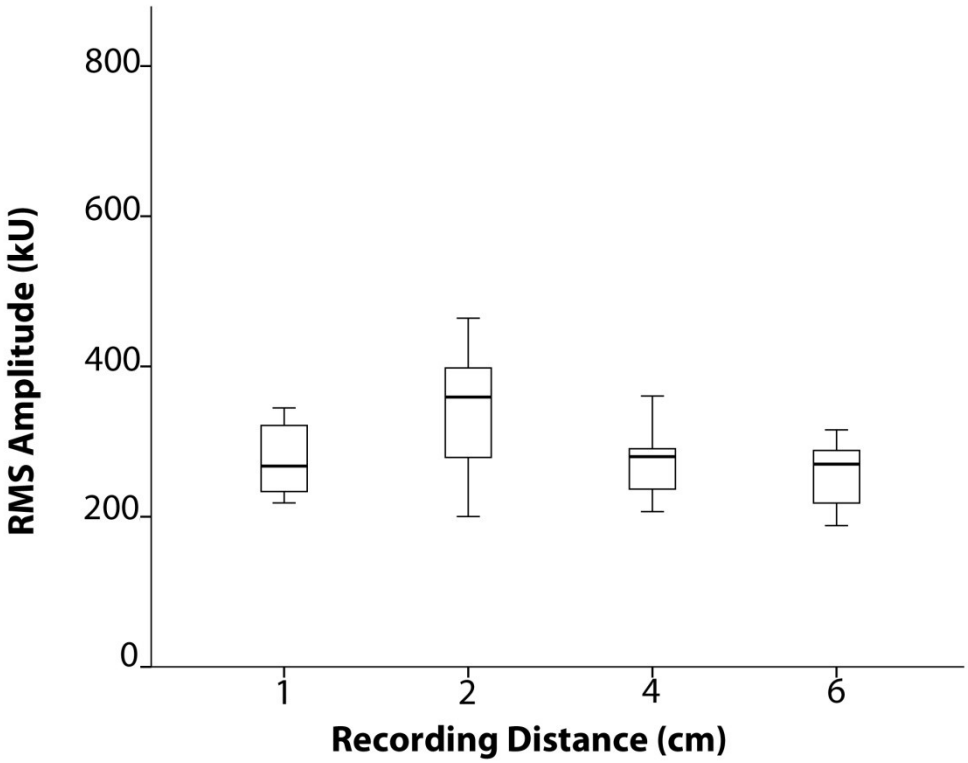

Figure 2.20 Experiment 3: Comparison of anal scrape mean RMS amplitude (kU) between four different recording distances.

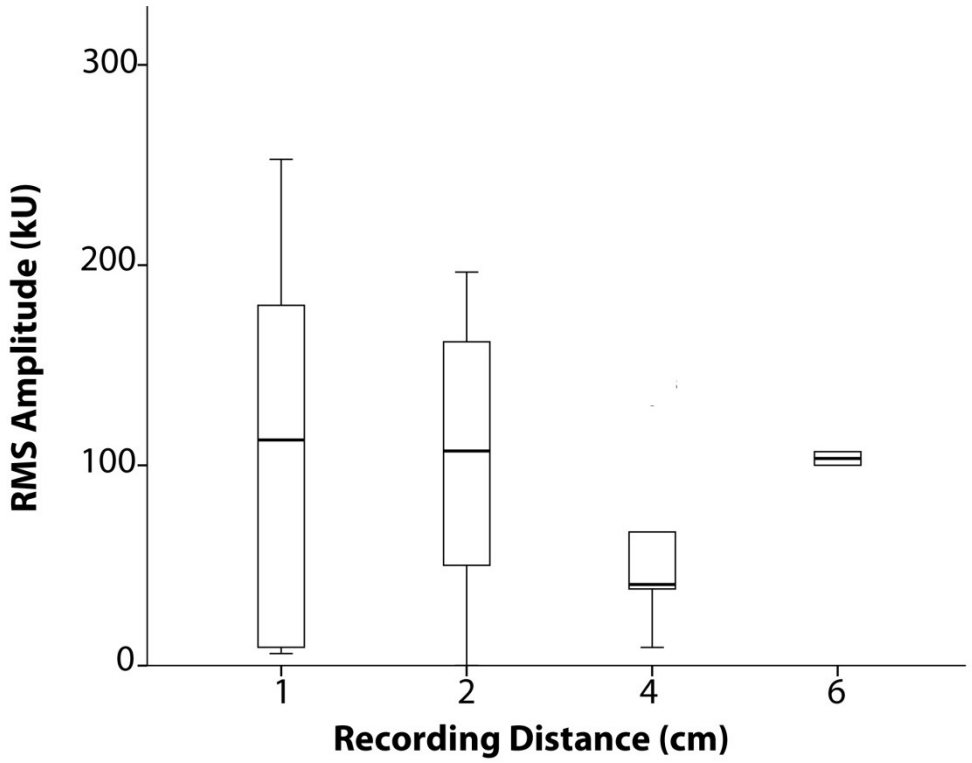

Figure 2.21 Experiment 3: Comparison of mandible drum mean RMS amplitude (kU) between four different recording distances.

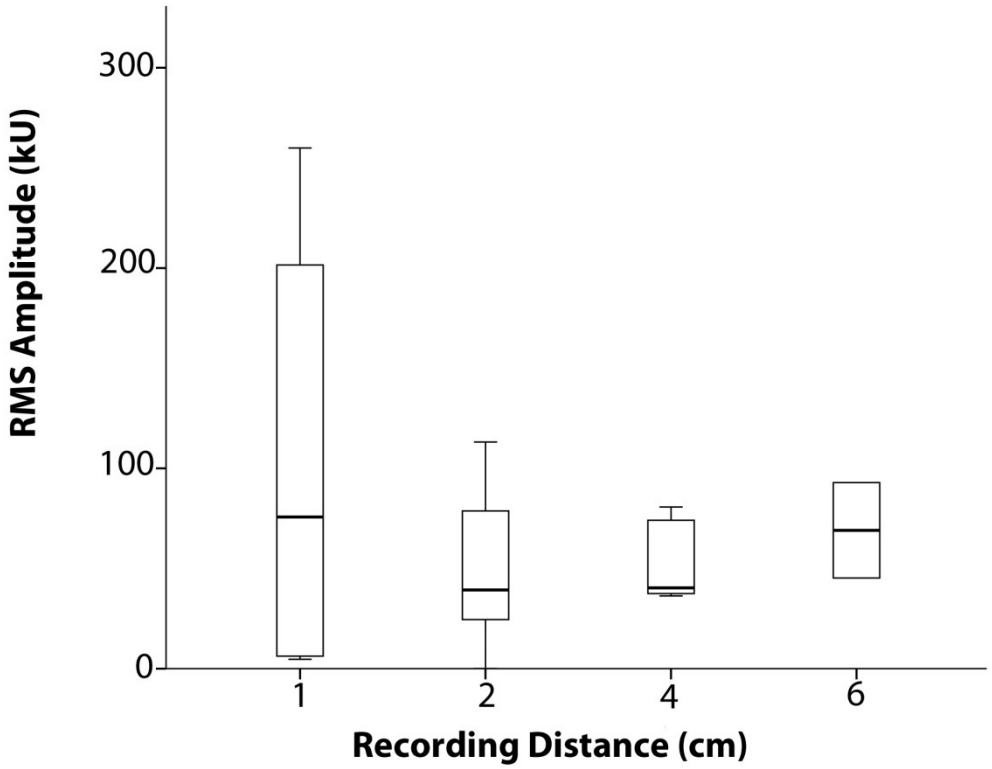

Figure 2.22 Experiment 3: Comparison of mandible scrape mean RMS amplitude (kU) between four different recording distances.

Discussion

The use of vibration communication by lepidopteran larvae for defending territories has been documented in a number species (Yack et al. 2001, Fletcher et al. 2006, Bowen et al. 2008, Scott et al. 2010, Guedes et al. 2012, Yack et al. 2014). Each species varies in the number of signals they use when defending their territory (Yack et al. 2001, Fletcher et al. 2006, Bowen et al. 2008, Scott et al. 2010). The use of multiple signals suggests that each confers different information. The hypothesis for experiment 1 of this study was the 3 signals produced by *D. arcuata* conveyed different meaning, the prediction was that the 3 signals would vary in their signal characteristics. Significant differences were observed for duration with anal scrapes being longer than both mandible drums and scrapes which could be due to how these signals are produced. Anal scrapes are produced by dragging the abdomen in the anterior direction while mandible drums are produced by striking the leaf surface and mandible scrapes are produced by a fast lateral movement of the head. When the peak amplitudes of anal scrapes and mandible drums were compared they did not vary significantly. A significant difference was observed when comparing the peak amplitudes of the mandible drums and scrapes. Mandible scrapes are the final signal to be incorporated into a territorial display perhaps the transition from using only mandible drums to incorporating mandible scrapes in a display is important to the interaction. RMS amplitude refers to the average amplitude of the signal, mandible drums and anal scrapes were higher in amplitude than mandible scrapes.

The results of experiment 1 do support the hypothesis that the 3 signals have differing signal characteristics specifically with respect to duration, peak amplitude and RMS amplitude. The caterpillars may use the differing characteristics to determine what type of signals are being used in a display. Anal scrapes can be distinguished from the other 2 signals by duration.

Mandible drums and mandible scrapes can be identified due to their difference in peak amplitude. This difference may be important during a display, indicating the incorporation of the mandible scrape. Finally RMS amplitude can be used to identify the mandible scrape from the other 2 signals in a display. The results of experiment 1 suggest that the caterpillars use 3 distinct signals to confer different information. They may also use different signal characteristics to identify each signal within a display. A behavioural experiment monitoring the intruder's response when the sound producing structures are inhibited will determine what kind of information may be conferred during an interaction.

Experiment 1 determined that signals have different characteristics. Experiment 2's goal is to determine whether the signals differ in relation to content specifically size. When comparing between signals significant differences were observed in the characteristics of the mandible drum and scrape however no differences were observed in anal scrapes. This was an unexpected result since the duration of the anal scrape was assumed to increase with body length. As the caterpillars increase in body length this would lead to an increase in the distance over which they scrape the leaf. There are two reasons related to morphology that could explain why there was no observable difference in anal scrape duration. One is that the anal scrape is produced over a fixed distance. Another explanation is that smaller caterpillars scrape over a longer distance. Comparing videos of anal scrape production by large and small caterpillars would determine whether anal scrapes are produced over a fixed or variable distance. An alternative explanation is that the lack of variation is due to the content of the signal as opposed to morphology. In several anuran species some signal parameters such as pulse rate are static conveying species identification while others such as pulse number are dynamic and reflect different aspects of the signaler (Gerhardt 1991). The anal scrapes may be used to signal static

properties such as species or to indicate a territory is occupied while the other two signals indicate dynamic properties such as size. Mandible drums and mandible scrapes could convey size information. As the caterpillar's mass increased mandible drums were longer in duration and higher in velocity while the peak and RMS amplitude of mandible scrapes increased. Since larger intruders have been observed to sometimes displace a resident from its territory it is likely that size information is exchanged during a display (Yack et al. 2001). In wolf spiders, *Hygrolycosa rubrofasciata*, and meadow katydids, *Conocephalus nigropleurum*, size information is transmitted through signal rate, larger individuals have lower signal rates than smaller individuals (Kotiaho et al. 1996, 1998, De Luca and Morris 1998). Mandible drums can confer size information due to the way the signal is produced, by lifting the head and thorax and striking the leaf surface, thus a heavier individual can produce a signal that is both longer and higher in velocity. The drum duration of male wolf spiders indicates male quality (Parri et al. 2002). Mandible scrape peak amplitude increased as mass increased and this could be due to how the signal is produced, a lateral sweeping motion of the head. A heavier individual may be able to generate more pressure between the mandibles and leaf surface thus creating a signal with higher peak amplitude.

The results of experiment 2 support the hypothesis that signals vary with size, significant changes in signal characteristics were observed when comparing between individuals of differing masses. Anal scrape duration did not vary between different sized individuals which suggests it may be a static signal conveying information such as species identity or occupation of a leaf. Both mandible drums and mandible scrapes can confer size information and each signal can be differentiated using duration and peak amplitude and RMS amplitude. The mandible scrape is produced when the contest has escalated, caterpillars that have longer residency durations are

more likely to produce mandible scrapes (Yack et al. 2014). The mandible scrape may confer motivation however this was not tested for in this experiment. These results suggest that the 3 signals used by *D*. *arcuata* relate to content. Size may not be the only information transmitted by these signals, further experiments are required to discern what other pieces of information is conferred during an acoustic display.

The hypothesis of experiment 3 was that the 3 signals have different transmission properties and that signal characteristics would vary at the different recording distances. This relates to the efficacy based hypothesis, if the 3 signals of *D. arcuata* show significant variation between the measured characteristics at different distances this suggests that each is used at different stages of an interaction due to their efficacy of traversing the environment. The only significant differences were observed between anal scrape duration and peak amplitude which could be due to the transmission properties of the leaf. A number of trends could be seen all 3 signals showed an increase in duration at 2 cm which suggests that this may be the optimal distance for communicating. The peak amplitude of the mandible drum increased with distance with values being higher at 6 cm than values at 1 cm. The increase in mandible drum amplitude is unexpected but it could be due to the difference in transmission properties of the soft leaf tissue and the harder petiole which is where the 6 cm laser disc is placed. Leaf structure has been shown to affect signal characteristics in terms of energy loss and frequency reduction (Magal et al. 2000). Amplitude reduction has been characterized in a study on signal propagation along a branch (McVean and Field 1996). The caterpillars may produce signals that match the resonance properties of the leaf which may explain why little variation was observed between signal types. The southern green stinkbug, *Nezara viridula*, produces vibration signals that match the resonant properties of the plant thus there is minimal amplitude and frequency attenuation as the signal is

transmitted through the plant (Cokl et al. 2005). In Guedes' study (2012) the leaf vibrations were characterized, the dominant frequency of the leaf was lower than dominant frequencies of all the signals produced by *D. arcuata*. This indicates that the transmission properties of the leaf are unlikely to interfere with the transmission of the signal. Although minor trends were observed in the results of experiment 3 they did not support the hypothesis, anal scrape duration and peak amplitude showed significant differences however the other signals did not. More work needs to be done in order to determine whether the signals of *D. arcuata* differ in their transmission properties.

This portion of the study was focused on determining the meaning of the 3 signals. Three experiments were devised to determine whether the signals differed between each other and whether the differences were due to content or efficacy. Experiment 1 determined that each signal differed between each other with respect to specific characteristics suggesting each conveyed different information. The second experiment aimed at determining whether the signals characteristics varied with size. The duration and velocity of the mandible drums as well as the peak amplitude of the mandible scrapes increased with caterpillars of larger size suggesting each may confer size information. The third experiment looked at whether the 3 signals and their measured characteristics varied with distance. Only a few differences were observed suggesting the 3 signals are equally efficient when transmitted through the leaf however further testing is required to confirm this.

Chapter 3

Putative vibration receptors in the masked birch caterpillar, *Drepana arcuata* (Drepanoidea: Drepanidae)

3.1 Acoustic Receptors in Insects

The previous chapter has shown that *D.arcuata* has complex signal patterns. Guedes et al. (2012) demonstrated that these caterpillars can detect vibrations in a noisy environment. An outstanding question is how they detect vibrations. In this chapter I will provide some preliminary evidence for putative receptor organs. First, I will review the basic receptor types with a few examples in adult insects. Then, I will describe the proposed receptors in immature insects. Finally I will discuss the findings of my morphological study on the proposed receptor of *D. arcuata*.

Acoustic communication plays an important role in the lives of insects, therefore they must possess structures that are responsible for detecting acoustic signals. Acoustic signals are produced by changing air pressure, water pressure or through substrate displacement (Greenfield 2002, Virant-Doberlet and Cokl 2004, Cocroft and Rodriguez 2005). Acoustic receptors are sensitive to these changes and vary in their location on the body of an insect (Yager 1999, Yack 2004). The receptors can be separated into two broad categories: particle or pressure detectors (Yager 1999, Yack 2004, Sanborn 2008). A particle detector is typically an elongated structure which bends when impacted by particles, the movement of the structure elicits firing from sensory cells located at its base. A pressure detector is a membrane that bends when pressure is unequal on the two sides of the membrane (Sanborn 2008). In the next section I will provide an overview of acoustic receptors in adults and proposed acoustic receptors in immature insects.

3.1.2 Acoustic Receptor Types

There are four main types of insects acoustic receptors: trichoid sensilla, Johnston's organ, subgenual organ and tympanal organ (Yager 1999 and Yack 2004). Trichoid sensilla are innervated hair-like cuticular projections that respond to near field sounds and are found in some species of lepidopteran caterpillars (Markl and Tautz 1975). Johnston's organ is associated with the antenna and also detect near field sounds (Yack 2004). Johnston's organ has been described in the antennae of a mosquito species, *Aedes aegypti*, as well as a leaf hopper species, *Oncopsis flavicollis*, and in honeybees, *Apis mellifera* (Howse and Claridge 1970, Saeng Boo and Richards 1975, Dreller and Kirchner 1993). The subgenual organ is a vibration receptor and differs in structure between insect groups and is usually located at the tibia of the legs (Virant-Doberlet and Cokl 2004, Yack 2004). Subgenual organs have been characterized as vibration receptors in green lacewings, *Chrysoperla carnea*, carpenter ants, *Camponutas ligniperda*, and honey bees, *Apis mellifera carnica* (Menzel and Tautz 1994, Sandeman et al. 1996, Devetak and Amon 1997). Tympanal organs are the most complex of insect auditory receptors and detect far field sounds and consist of 3 parts: a tympanal membrane, tracheal sac and a chordotonal organ (Yack 2004). Tympanal organs have been characterized in a number of insect species across a range of orders including Coleoptera, Dictyoptera, Diptera, Hemiptera, Lepidoptera, Neuroptera and Orthoptera (Hoy and Robert 1996, Yager 1999, Yack 2004). Acoustic receptors in adults have been extensively reviewed but much less is known about how immature insects detect sounds and vibrations (Howse 1968, Yager 1999, Gerhardt and Huber 2002, Yack 2004, Chapman 2013).

3.2 Acoustic Receptors in Immature Insects

There are few descriptions of acoustic receptors in holometabolous insect larvae. Larval vibration receptors that have been described include specialized structures, modified setae and antennae. These structures vary in their location on an insect's body and serve different functions. Most of the proposed structures have not been tested using behavioural experiments or electrophysiology.

3.2.1 Pleural Discs

A morphological study on cerambycid larvae resulted in the discovery of ray like structures in the abdominal region termed pleural discs (Hess 1917). The pleural discs are thought to play a role in orientation of the larvae (Saliba 1972). Some larvae also possessed modified pleural discs in an elliptical arrangement called a pleural tubercle (Hess 1917). A chordotonal ligament attaches to the centre of the disc and attaches to an anterior body fold (Hess 1917). The chordotonal ligament attaches to eight abdominal segments; each ligament contains four scolopophores (Hess 1917). Each scolopophore is composed of a sense cell covered at its distal end by two enveloping cells; the distal portion of the sense cell penetrates the center of the enveloping cell, where the axis fibre of the sense cell enlarges to form the scolopale. The scolopales are arranged so that none were the same distance from the posterior (Hess 1917). In Saliba's study (1972) the larvae were observed to re-orient themselves when exposed to the vibrations produced by conspecifics gnawing on wood. Whether the response was due to the activity of the pleural discs was not confirmed.

Table 3.1 Summary table of putative vibration receptors in immature insects

Insect taxa	Vibration receptor	Location	Function	Reference
COLEOPTERA Cerambycidae,	Pleural disc	Abdominal pleural region	No proposed function	Hess 1917
LEPIDOPTERA Arctiidae, *Isia isabella*, Liparidae, *Liparis dispar*, Lasiocampidae, *Malacosoma disstria*, Geometridae, *Cingilia catenaria*, Pieridae, *Pieris rapae* and Nymphalidae, *Nymphalis antiopa* ODONATA Coenigrionidae, *Erythromma lindenii*	Antennal receptor	Head	Mechanoreceptor, proprioception, olfaction	Dethier 1941, Meurgey and Faucheux 2006
LEPIDOPTERA Pyraloidea, Gelechioidea	Web-vibration receptor	Mesothorax, abdominal segments	Detect web vibrations	Hasenfuss 1992
LEPIDOPTERA Noctuidae, *Barathra brassicae*, Lymantriidae, *Orgyia leucostigma*	Hair receptor	Thoracic segments	Detect substrate vibrations, near field airborne sound	Markl and Tautz 1975, Castellanos et al. 2011
LEPIDOPTERA Drepanidae, *Drepana arcuata*	Setae and chordotonal organs	Proleg	Detect substrate vibrations produced by conspecifics	Hasenfuss unpublished data

3.2.2 Setae

Setae stimulated by vibrations have been demonstrated to play a role in triggering predator defense tactics in two species of larval Lepidoptera (*Barathra brassicae*, Markl and Tautz 1975, *Orgyia leucostigma*, Castellanos et al. 2011). The cabbage moth caterpillar, *B. brassicae*, possesses eight filiform setae on the dorsal surface of its thoracic region. Each hair is innervated by a sense cell; reception threshold increased as the number of intact hairs decreased (Markl and Tautz 1975). Larvae responded to low frequency stimuli by dropping, squirming or stopping; the hairs are thought to function in detecting the wing vibrations produced by parasitic wasps and flies (Markl and Tautz 1975). Setae serve a similar purpose in *O. leucostigma*, Castellanos et al. (2011) demonstrated that the larva's response was dependent on the characteristics of the vibration. Larvae were observed to drop if the hair was stimulated by high hair bending velocities produced by parasitic wasps and walk away in response to low hair bending velocities produced by stink bugs (Castellanos et al. 2011). The responses to each type of stimuli resulted in increased survival of the larvae (Castellanos et al. 2011).

Hasenfuss (1992) characterized larvae of butterflies belonging to the superfamilies Pyraloidea and Gelechioidea which possessed elongated setae which have a short apodemum at their origin connecting to a chordotonal organ. The setae and the apodemum function as a system of levers transmitting substrate-borne vibrations to the chordotonal organ (Hasenfuss 1992). The larvae of these two families usually construct tubular silk webs; when inside the setae are bent and remain in contact with the threads of silk in the wall (Hasenfuss 1992). Vibrations may be detected by the setae in contact with the web and through the seta-apodemum lever system these

vibrations may be transmitted to the chordotonal organ (Hasenfuss 1992). The function of the setal-apodemum system has yet to be established experimentally.

3.2.3 Antennae

The antennae of larval Lepidoptera are located on the ventral surface of the head near the base of the mandibles; each antenna is three-segmented (Dethier 1941). The first segment contains four sensilla campaniformia while the second segment has a sensillum campaniformium two sensilla trichodea and three sensilla basiconica (Dethier 1941). The third segment consists of four apical sensilla: a sensillum styloconicum, a large sensillum basiconicum, and two smaller sensilla basiconica (Dethier 1941). The antennae were observed to move in their sockets when stimulated with a glass rod (Dethier 1941). Larvae of goblet-marked damselfly, *Erythromma lindenii*, possess antennae comprised of a scape, pedicel and a four segmented flagellum (Meurgey and Faucheux, 2006). The flagellum bears four mechanoreceptive sensilla: spatula-shaped sensilla chaetica, curved sensilla chaetica, sensilla filiformia and sensilla campaniformia (Meurgey and Faucheux, 2006). The spatula-shaped sensilla chaetica are tactile receptors while the curved sensilla chaetica are proprioceptors that monitor the position of the 3rd and 4th flagellomeres (Meurgey and Faucheux, 2006). The sensilla filiformia are vibration receptors which are involved in detecting prey and changes in water pressure and the sensilla campaniformium is a proprioceptor informing the larvae of the location of the flagellum in relation to the pedicel (Meurgey and Faucheux, 2006). The primary sensilla are the sensilla filiformia, the other sensilla serve to enhance the abilities of the sensilla filiformia (Meurgey and Faucheux, 2006).

3.3.1 Putative Receptor Locations in D. arcuata

Earlier studies were conducted on internal and external morphology of *D. arcuata*. A morphological study was conducted on the prolegs of the caterpillar by Hasenfuss. Hasenfuss provided detailed drawings of the external and internal structures of the proleg as well as the nerve branches which innervated these structures. An undergraduate research project conducted by Tamara Neville focused on the external structures of the caterpillar. She determined that the SV1 and SV2 setae contact the leaf surface and proposed they may play a role in vibration detection. I conducted an undergraduate research project on the prolegs using microdissections and scanning electron microscopy (SEM). The SEMs provided detailed images of the external morphology (Fig 3.1).

To determine how *D. arcuata* sense vibrations a list of proposed receptors and their locations should be considered. It may be possible that the larvae possess chordotonal organs; which can be distributed throughout the insects body (Yack 2004). The setae may be sensitive to vibrations if associated with sense cells or chordotonal organs. The larvae may possess leg scolopidial organs in their thoracic legs or abdominal prolegs. Another possibility is a chordotonal organ associated with the setae, Hasenfess (1992) described thoracic setae associated with a chordotonal organ through an apodemum present in members of the superfamilies Pyraloidea and Gelechioidea. Masked birch caterpillars construct silken shelters, they may detect vibrations through the vibrations transmitted through the silken strands composing their shelter which in turn stimulate their setae. The locations of the receptor were considered and subsequently ruled out using the observations of behavioural experiments.

The prolegs were considered as a location for vibration receptors organs because they are in contact with leaf surface constantly. The caterpillars are known to assume an S-position when

they are not feeding or building a shelter; this position is characterized by the head and thorax being lifted off the leaf surface and curled while its abdomen still contacts the leaf. Despite being in this position the larva is still able to detect vibrations suggesting that the prolegs may play a role in vibration detection; this was confirmed by video analysis. The prolegs of *D. arcuata* also possess setae which contact the leaf surface similar to the organization of the setae of *M. sexta*. The proleg hairs may be innervated and could potentially play a role in detecting vibrations (Neville 2010, Nathan 2011).

A goal of this portion of the study is to determine whether the proleg is innervated by the neural ganglion through microdissections. Another goal of the microdissections is to determine whether the larvae rely on one receptor located within the proleg or whether there are other components that function together to receive vibrations.

3.4 Methods

3.4.1 Insects

Caterpillars ranging from third to fifth larval instar were fixed in either alcohol, glutaraldehyde or Bouin's solution. All the solutions were introduced using the same procedure, a hypodermic needle was filled with solution which was then inserted into the body cavity. The insertion point of the needle was on the ventral surface between the 2nd and 3rd abdominal segments. The caterpillar was filled with solution until it was slightly inflated indicating a sufficient amount had been introduced. The fixed caterpillars were then stored in vial containing the same solution. 3 caterpillars from each fixing solution were dissected for a total of 9 caterpillars used.

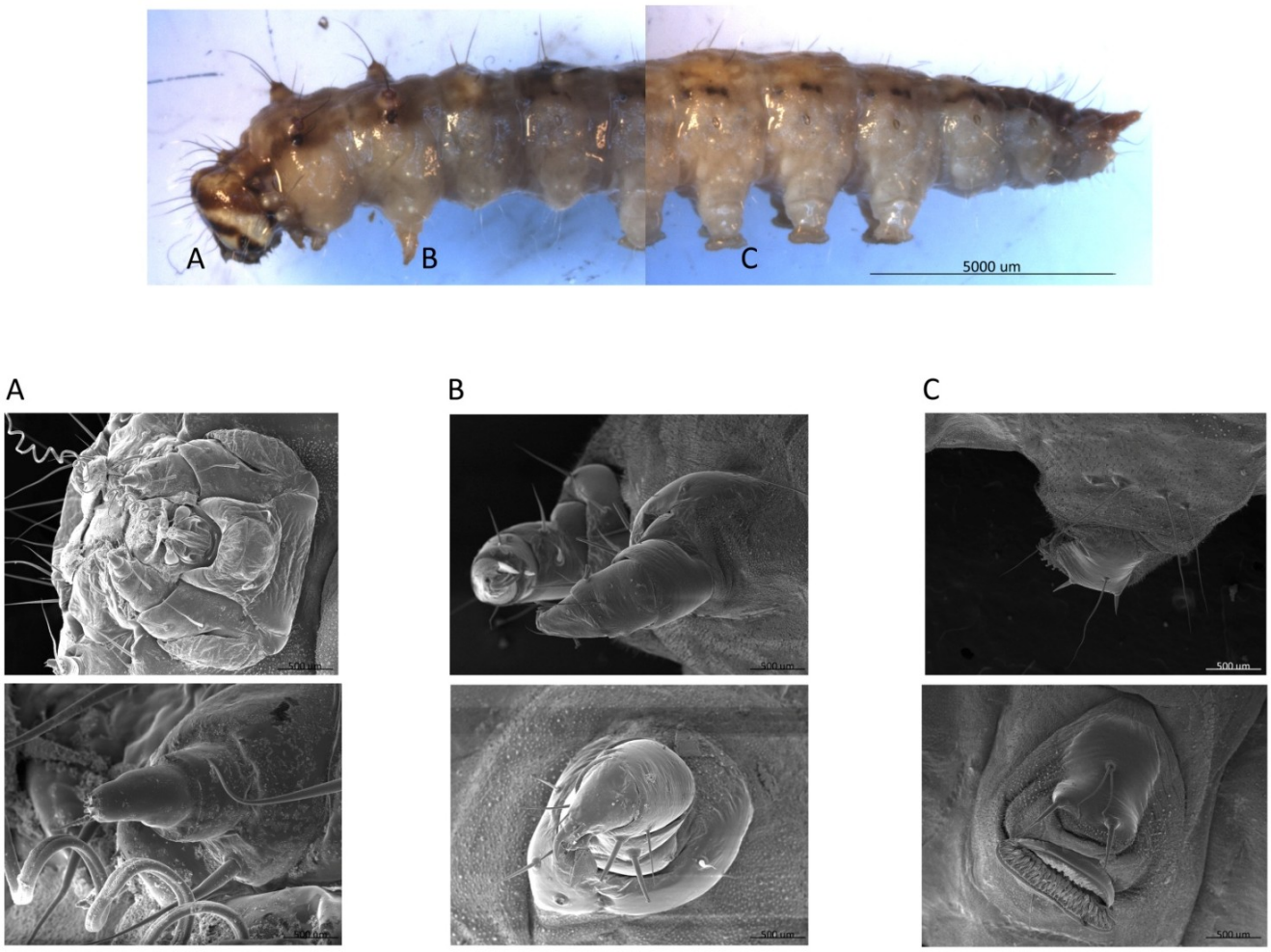

Figure 3.1 External morphology of late instar caterpillar, *D*. *arcuata*. Full length of late instar caterpillar, scale bar 5000 µm. (A) Ventral view of the head of a late instar caterpillar, scale bar 500 µm. Close-up of larval antenna, scale bar 500 µm. (B) Lateral view of the thoracic legs of a late instar caterpillar, scale bar 500 µm. Ventral view of a thoracic leg, scale bar 500 µm. (C) Lateral view of proleg, of a late instar caterpillar scale bar 500 µm. Ventral view of proleg scale bar 500 µm. Scanning electron micrographs (SEM) taken by Peter Gordon. SEMs performed during honour's thesis.

3.4.2 Dissection

Microdissections were conducted on several specimens and diagrams provided by Hasenfuss (personal communication) were used as a guide in addition to diagrams from *The Lepidoptera: Form Function and Diversity* by Malcolm J. Scoble (1992) as well as the nerve branch maps from *Lepidopteran Anatomy* by John L. Eaton (1988).

The caterpillar was bisected and an abdominal segment was isolated, a central incision was made through the dorsal surface, the proleg body wall on either side were held open with minutien pins. Light micrographs were taken using an Olympus (Tokyo, Japan) SZX12 light microscope equipped with a Zeiss (Oberkochen, Germany) AxioCamMRc5 camera (1.4 mega pixels, 1388 x 1040). Janus Green B was used to visualize the central ganglion and nerve branches (Yack 1993). The nerve branches were followed to determine their point of attachment and whether these corresponded with the diagram provided by a colleague, Hasenfuss. Another dissection method involved making a lateral incision on the outer surface of the body wall, slowly the outer body wall was peeled proceeding down towards the proleg. Janus Green B was used to visualize the afferent nerve branches.

3.5 Results

A drawing from Eaton's book depicted the abdominal central ganglia of the caterpillar and identified the N2 branch as the main nerve branch which innervated the muscles and sensory sensilla of the ventral region (Fig 3.2). Four separate attempts to follow the N2 branch from the abdominal central ganglion into the proleg yielded no results so a direct dissection of the proleg was employed. The internal anatomy of the proleg was observed for the first time using the

direct approach (Fig 3.3A). Hasenfuss' drawing was used to identify these branches (Fig. 3.3B). The main branch labelled Np was not seen, Np was depicted being thicker that the other branches suggesting it could be a projection of N2. The Np branch descends into the proleg and a single projection labelled Nps (green arrow) splits into numerous branches that innervate structures within the proleg. In Hasenfuss' diagram Nps directly innervates the chordotonal organ Scsv, however, this was not observed in the dissection preparation. The structure Scsv may have been dislodged or severed when dissecting the proleg directly. A short Nps branch bifurcates with one branch innervating the inner wall of the proleg (purple arrow) and the other innervating the outer wall (red arrow). The branch that innervates the inner wall connects to the V1 seta and the chordotonal organ Scv. The outer wall branch splits into three smaller branches which innervate the SV1 (red arrow), SV2 (black arrow) and SV3 setae (blue arrow).

A tubular structure was observed descending towards the base of the proleg. In Hasenfuss' diagram, a similar structure is seen in the same orientation labelled Scsv descends into the proleg, he identified this structure as a chordotonal organ. The structure was attached to the proleg body wall and descended towards the base of the proleg. A second chordotonal organ, Scv, was described in Hasenfuss' figure situated at the base of the proleg. The structure was not observed in the dissection preparation. The main shaft of the nerve branch (purple arrow, Fig 3.4) which supposedly innervates the structure was observed. The SV1 and SV2 setae, which contact the leaf surface, were associated with structures that resembled bipolar type 1 cells which suggest the setae's involvement in vibration detection. The structure and setae may function to process particular characteristics of a signal suggesting the possibility of a complex receptor system. Further dissections are required to confirm whether the structure is innervated or is associated with scolopidia to determine its role in vibration detection.

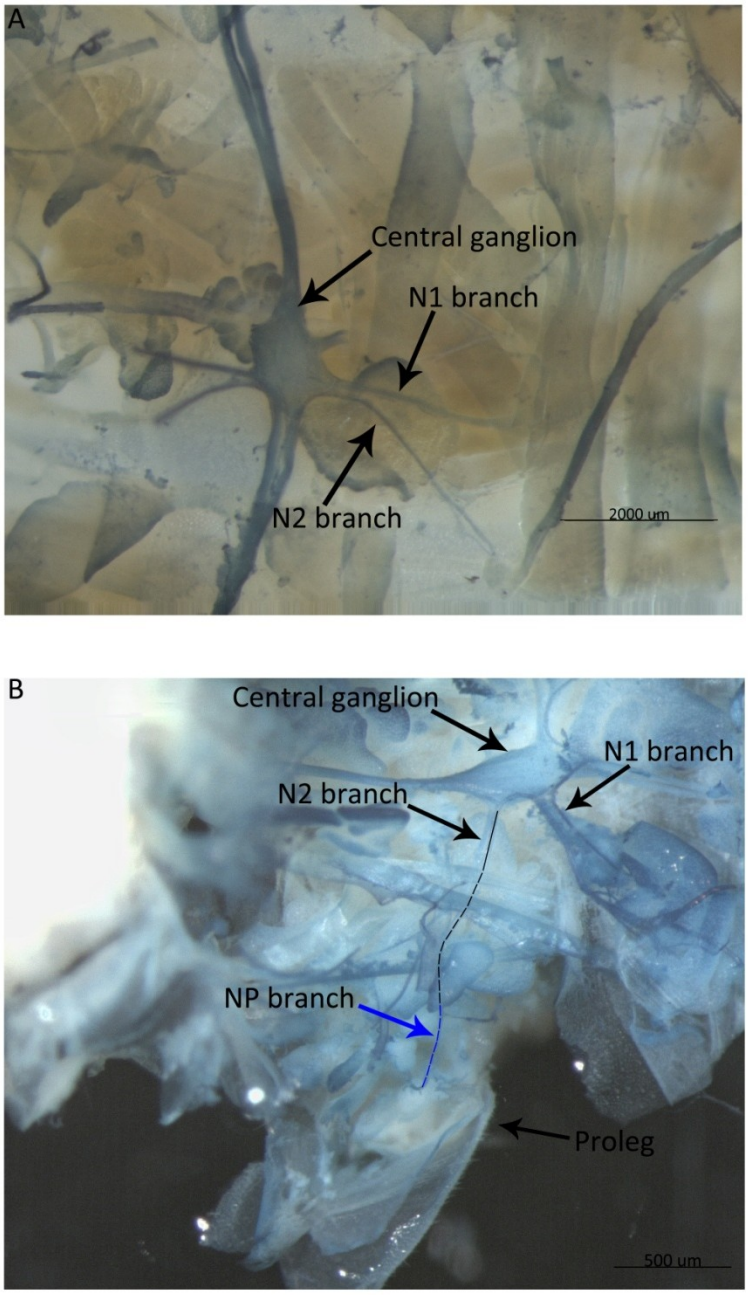

Figure 3.2 Photograph of the abdominal central ganglion of *D. arcuata*. (A) View of the central ganglion and the N1 and N2 branch, scale bar 2000 µm. (B) Trace of N2 branch path towards the proleg as well as NP branch which descends into the proleg, scale bar 500 µm.

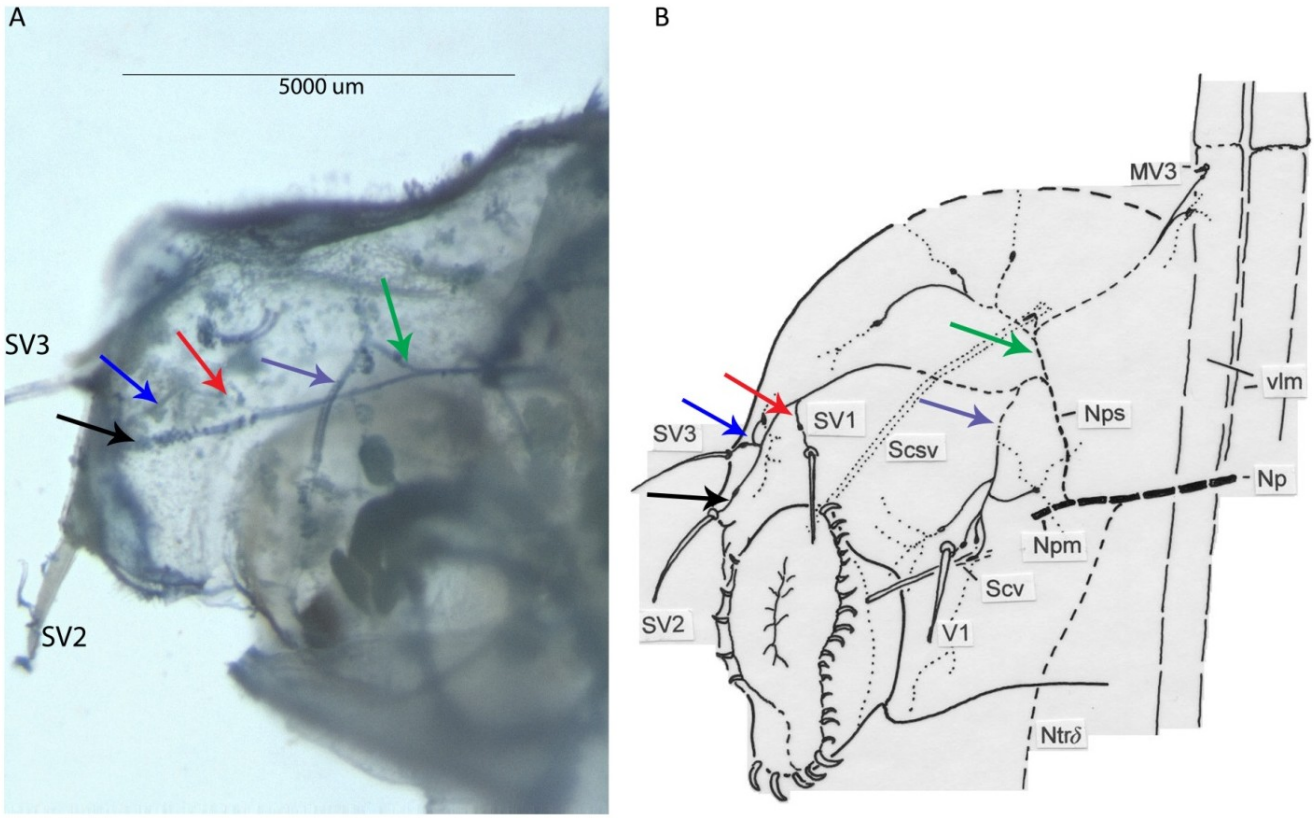

Figure 3.3 Comparison between dissection preparation of proleg of *D. arcuata* and diagram of internal proleg anatomy by Dr. I. Hasenfuss. (A) Photograph of dissection proleg, scale bar 5000 µm. Black arrow indicates nerve branch innervating SV2 seta. Blue arrow indicates nerve branch innervating SV3 seta. Red arrow points to nerve branch innervating SV1 seta. Purple arrow indicates Nps branch which innervates lower regions of proleg. Green arrow indicates main Nps branch which innervates proleg body wall. (B) Diagram of internal proleg anatomy. Two chordotonal organs are depicted labelled Scsv and Scv. Scsv is innervated by the main Nps branch while Scv is innervated by afferents of the second Nps branch which descends into the proleg.

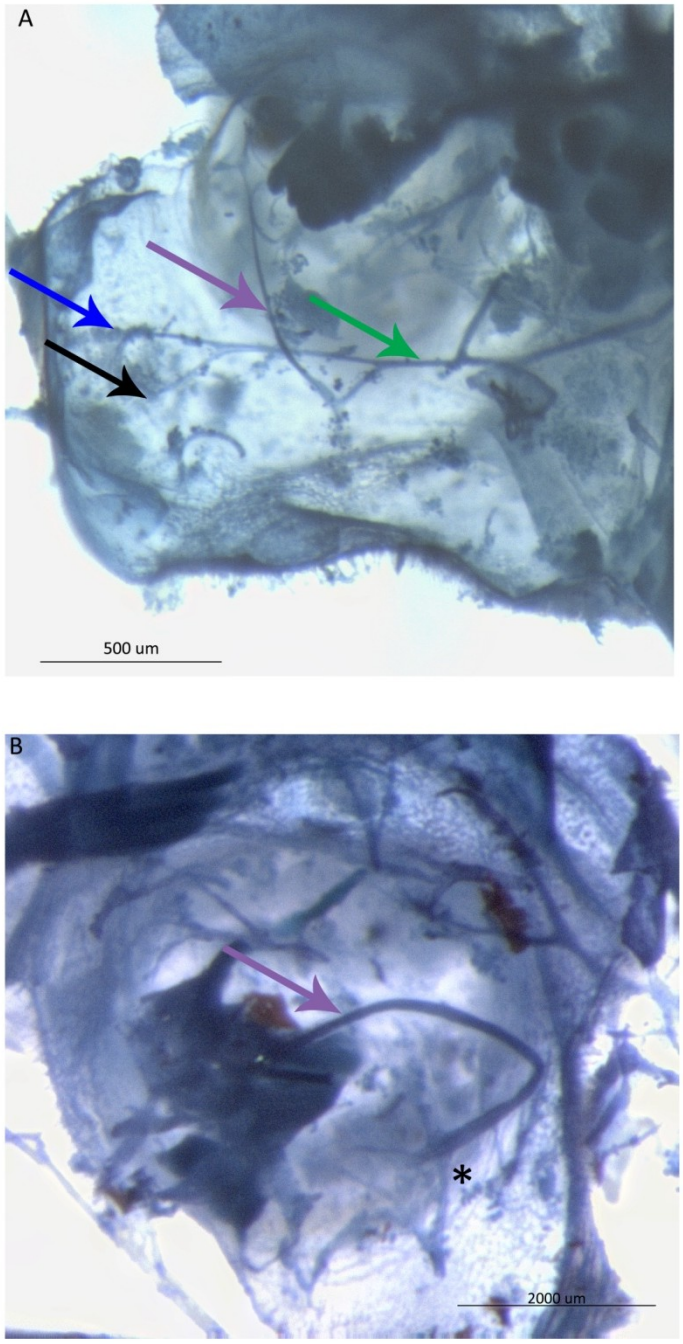

Figure 3.4 Proleg dissection of *D. arcuata*. (A) View of Nps branch, scale bar 500 µm. Green arrow indicates main Nps branch. Purple arrow indicates second branch of Nps which descends into proleg. Black arrow indicates Nps branch innervating SV2 seta. Blue arrow indicates Nps branch which innervates SV3 seta. (B) View of second Nps branch descending into proleg, scale bar 2000 µm. Asterisk marks a structure which may be Scv, one of the chordotonal organs depicted in Hasenfuss' diagram.

3.6 Discussion

There appears to be a complex receptor system within the proleg of *D. arcuata*. The proleg is innervated, a main nerve branch Np descends into the proleg and gives rise to the Nps branch which in turn has many projections. These projections split and attach to external or internal proleg structures namely the setae and 2 chordotonal organs. The SV1 and SV2 setae may play a role in vibration detection since they contact the leaf surface and are innervated. The 2 putative chordotonal organs Scsv and Scv are both tubular structures which are innervated by Nps projections. Scsv is longer, originating higher up within the proleg where it is innervated directly by the Nps branch. Scv is a much shorter structure that attaches to the inner surface of the body close to the V1 seta and descends into the proleg. Scv is innervated by an Nps projection which also attaches to the inner proleg body wall and V1 seta. The function of these structures still needs to be confirmed through behavioural and electrophysiological experiments.

We now have new information about the internal anatomy of the proleg of *D. arcuata* which includes knowledge on specific nerve branches and the structures they innervate. The proleg of the Tobacco Hornworm, *Manduca sexta*, was described as a complex sensory system which was highly innervated and had external sensory organs, the setae (Kent et al. 1996). The prolegs of the two species share some morphological similarities. The location and organization of the setae is similar the only difference is that *M. sexta* has many planta and lateral hairs while *D. arcuata* only has 3 setae in that location, SV1, SV2 and SV3. The hairs of *M. sexta* play a role in movement, the hairs of *D. arcuata* may play a similar role however *D. arcuata* does communicate using substrate vibrations and since the SV1 and SV2 setae contact the leaf surface they may play a role in reception. Previous studies have shown that caterpillar prolegs are complex structures which are extensively innervated and possess accessory structures that

function in proprioception and movement (Belanger et al. 2000, Simon and Trimmer 2009, van Griethuijsen and Trimmer 2010). Since these structures are activated by mechanical displacement they may play a role in vibration detection in species that use vibrations to communicate such as *D. arcuata*.

Vibration receptors have yet to be characterized in a larvae of a holometabolous insect species that uses vibrations for communication. As discussed previously, *D. arcuata* use three signals when communicating; signal rate and the type of signal used have been shown to change under different experimental conditions (Yack et al. 2001, Guedes et al. 2012, Yack et al. 2014). Guedes et al. (2012) demonstrated that amplitude and frequency characteristics were important when determining whether a vibration source was relevant or non-relevant. He also suggested that vibration receptors in *D. arcuata* are tuned to higher frequencies to avoid interference (Guedes et al 2012). In chapter 2 of this study it was observed that each signal differed in terms of duration, peak amplitude and RMS amplitude which could be used to distinguish between them. Furthermore there were differences in signal velocity and duration between individuals of different masses. The caterpillars must possess receptors that are able to detect and differentiate between these signals using their characteristics.

The setae and chordotonal organs of the proleg may work together to detect vibrations, there are few examples of a similar system. One example is the thoracic setae described by Hasenfuss (1992) where the setae connects to an apodemum which in turn attaches to a chordotonal organ however its function was not confirmed. The proleg setae of *D. arcuata* are composed of cuticle and seem to be rigid thus they are unlikely to pick up the near field vibrations that the filiform hair receptors of other species detect. The SV1, SV2 and V1 setae are innervated and all 3 contact the leaf surface, since these structures are rigid perhaps if held

against the leaf surface they can detect signal characteristics such as amplitude disturbances caused by the intruder walking on the leaf. According to Hasenfuss' work, *D. arcuata* seems to possess two chordotonal organs, Scsv and Scv, which is similar to the prothoracic chordotonal organ in the locust which is divided into the anterior chordotonal organ and ventral chordotonal organ. The anterior chordotonal organ had attachments to a tracheal system and acted as both an acoustic receptor and proprioceptor (Pflüger and Field 1998). Scv is located in close proximity to the V1 seta, Hasenfuss' diagram also shows the seta and organ are innervated by the same neural branch, the relevant signals that activate V1 seta sense cells could in turn activate the sense cells of Scv. These suggestions are hypothetical however given the evidence of the complex sensory role setae play in caterpillar proprioception and movement, it seems they may play an additional role in reception in species that use substrate-borne vibrations. The two chordotonal organs present in the prolegs is a novel reception system in immature insects, by studying the extensive innervation of the proleg it is evident many possible sense structures would be linked via the nervous system. This suggests the existence of a complex receptor system located in the prolegs comprised of the setae and the two chordotonal organs however further experimentation is required to confirm its function.

The morphological studies conducted on the prolegs of *D. arcuata* suggest that the innervated setae and chordotonal organs may function as receptors. To confirm their role further experiments need to be conducted namely histological, behavioural and perhaps electrophysiological experiments. Histology can confirm whether these structures contain a scolopidium, the unit that comprise a chordotonal organ, or cell types that comprise a scolopidium. The behavioural experiments will be useful for determining whether the setae are vibration receptors. Electrophysiological experiments are difficult to conduct on caterpillars due

to their bodies, including internal structures, being supported by hydrostatic pressure. Kent and Trimmer (1996, 2009) cooled the caterpillars in their studies and this prevented collapse of the body structure. Using this method we can conduct an electrophysiology experiment on the proleg of *D. arcuata* by using glass suction electrodes on the nerve branches that innervate the setae and chordotonal organs.

An alternative hypothesis to the setae and chordotonal organs playing a role in reception is that they play a role in movement and proprioception. The external anatomy of the proleg of *D. arcuata* does share some similarities to the proleg of *M. sexta* and could very well function similarly. The arrangement of the proleg setae is similar between the two species with *M. sexta* having more seta. Following the proleg setae brushing up against an object the caterpillar would adjust its proleg step trajectory (van Griethuijsen and Trimmer 2010). If an ablation experiment is carried out, the experimenter should take note of the movement of the caterpillar. The innervation of the proleg structures could all relate to the control and execution of movement. The nerve branches that innervate more than one structure may incorporate the different information provided by each to moderate movement. In order to confirm the function of the setae and chordotonal organs located within the prolegs of *D. arcuata* further experimentation is required, histology on the chordotonal organs.

Conclusion

The masked birch caterpillar, *D. arcuata*, produces complex signals when defending its territory from conspecifics. This study has provided new information on the meaning of these signals. Each of the 3 signals of *D. arcuata* differed with respect to 3 signal characteristics, duration, peak amplitude and RMS amplitude. The variation between the signals suggests they each have the potential to convey different meaning. When comparing between individuals of different mass the duration and velocity of the mandible drum as well as the peak amplitude of the mandible scrape were observed to increase when produced by larger individuals. The RMS amplitude of both the anal scrape and mandible drum increased as well. These findings suggest that the differences between the signals of *D. arcuata* are related to content. When the signals were measured from different distances the anal scrape duration and peak amplitude differed significantly. This variation was unexpected and further testing is required to determine whether the difference between signals is related to efficacy. In a morphological study conducted on the proleg of the caterpillar, the location of the nerve branches previously described by Hasenfuss were confirmed. The proleg setae and chordotonal organ may work together as a complex receptor system. The function of these structures has yet to be confirmed. This study has added to the existing knowledge base on *D. arcuata*, the different signal types are a result of content and the caterpillars possess putative receptors in their prolegs. Further experimentation is needed to determine the content of the signals, whether information other than size is transmitted by the signals, as well as the function of the proposed receptor system in the proleg.

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